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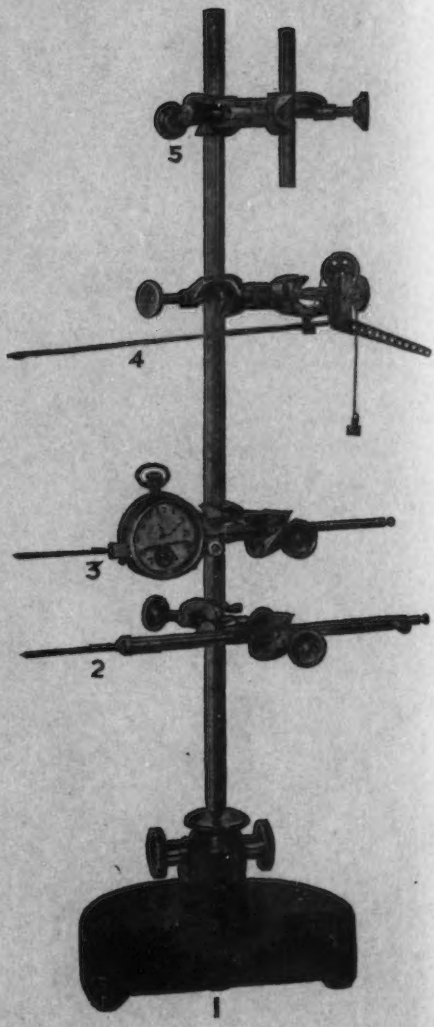

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THE INFLUENCE OF PHYSICAL TRAINING ON THE BASAL RESPIRATORY EXCHANGE, PULSE RATE AND ARTERIAL BLOOD PRESSURE

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There can be no doubt but that a regular course of physical training gives greater strength and efficiency to the body and improves its nutritive condition. Furthermore it is generally admitted that the trained man can perform a given amount of work with a smaller consumption of oxygen than the untrained; and that he, therefore, makes a smaller demand on his heart, with the result that it beats less frequently. Even during rest the heart beats less frequently in the trained than in the untrained man. We are taught (1), (3) that during rest there is some increase in the amount of oxygen consumed by the body of the trained man, because of the growth in the size of the muscles. Furthermore it is known that, as a rule, the pulse rate of all men rises and falls with the metabolism of the body. If the last two statements are true, we should expect the basal pulse rate to be slightly greater when a man is in training than when he is out of training.

It has been our purpose to investigate the relation of basal metabolism to pulse frequency and to attempt to follow step by step the basal changes in the gaseous metabolism, pulse rate and arterial blood pressure during a period of regular physical training and during an after-period when no exercise was taken, in order to determine how soon and to what extent regular exercise may change each. Our observations were made on one athlete, and on four men who were ordinarily very irregular in the matter of exercise and who, prior to the beginning of our study, had led a sedentary life. The training of the four required the spending of at least an hour every day in some form of vigorous physical exercise. In the fall this was mostly tennis and later consisted of running, handball and swimming. At times setting up exercises were employed. The period of training lasted from six to fourteen weeks. Our athlete played center on the varsity

football team and served as a subject during the football season and throughout the following winter months.

All observations were ordinarily made once a week in the early morning, the reclining observations just before the subject arose from bed and the standing immediately after arising. No unnecessary movements were allowed in the standing posture until after all observations had been completed.

For the basal metabolism determinations Douglas bags were used and the analyses for oxygen and carbon dioxide were made with a Haldane gas-analysis apparatus. The blood pressure was taken with a Tycos sphygmomanometer.

Basal metabolism. Heretofore observations on the influence of physical training on the basal metabolism have chiefly consisted of comparisons of trained with untrained men of about the same height and weight. The results obtained permit diametrically opposite conclusions. Thus Speck (11) found that muscular individuals had a greater respiratory exchange than non-athletic persons, while Magnus-Levy and Falk (10) found that three muscular individuals had the same or a slightly lower exchange than untrained persons. Benedict and Carpenter (2) found that a professional bicycle rider and three college athletes showed a uniformly low exchange per kilogram of body weight and per square meter of body surface. Later Benedict and Smith (3) compared fifteen athletes with thirty-three non-athletes. They found that the heaviest athletes almost invariably had a high basal metabolism; but that in those with a body weight under 65 kgm. it was not, with one exception, clearly high. None of their athletes showed a lower metabolism than the non-athletes. Their general conclusion was that athletes have a somewhat higher metabolism, both per kilogram of body weight and per square meter of body surface, than do non-athletes.

Zuntz and Schumberg (13) in their study of the effects of daily marching over a period of two and a half months recorded the oxygen used by two soldiers while at rest. The oxygen consumption of one of these men was not clearly altered in amount, while that in the other seemed to be increased. Zuntz (14) later published data of the resting metabolism of a dog, which showed an increase from 837 calories per day to 1,111 calories in four weeks of physical exercise.

More recently Lusk and DuBois (9) report of a dog that "cage life" in a darkened room reduced the basal metabolism from a level of 20 calories to one of 16.5 calories per hour. The basal metabolism of this animal was always higher in the autumn after an active life in sunshine and fresh air. Over against the above observations on the dog may be placed a study made on two dogs by Steinhaus (12). During one period the dogs were caged with practically no opportunity for exercise, and in another they ran

from two to twelve miles daily on a motor-driven tread. One animal showed an increase and the other a decrease in calories of heat eliminated per hour. Steinhaus points out that if the weight lost by the first dog was inactive fat deposits, then the increase in metabolism becomes insignificant; and that his data may be interpreted to show a lower metabolic rate in the muscles in the trained state.

Our own study of basal metabolism before, during, and after a period of regular physical exercise lends no support to the idea that the taking of daily exercise will increase the basal exchange of the body. It, on the

TABLE 1

Averages of basal metabolism observations

	W.F.A.		R.W.C.		A.L.H.		G.C.R.		E.C.S.	
	Out of training	Training	Out of training	Training	Out of training	Training	Out of training	Training	Out of training	Training
Respiratory minute-volume in liters	5.72	5.80	4.83	4.70	4.20	4.27	5.43	5.78	5.04	4.60
CO_2 exhaled per minute in cubic centimeters	245	241	189	179	188	194	201	198	187	177
O_2 absorbed per minute in cubic centimeters	293	289	230	216	222	235	231	227	221	208
Respiratory quotient	0.838	0.841	0.823	0.820	0.847	0.825	0.870	0.865	0.822	0.851
Calories per square meter per hour	45.0	43.1	36.5	34.3	36.4	36.7	39.3	38.7	36.8	34.3
Basal metabolic rate in per cent	+14.0	+9.5	−7.5	−13.3	−7.9	−7.1	−0.4	−2.0	−4.9	−10.5

whole, indicates that the basal gaseous exchange may be slightly reduced during a period of training. A summary of our data appears in table 1.

The largest number of determinations of the respiratory exchange was made on R. W. C. He is a tall, slender young man who may well be classed among the non-athletes. A total of eighteen determinations of his basal metabolism were made over a period of three and a half months before he began training. During this time his average metabolism was 36.5 calories per square meter of body surface per hour; and the basal metabolic rate, which is given in percentage above or below normal, averaged −7.5 per cent. His period of training, or of regular vigorous exercise, extended

from March 1 to June 6. In that time 28 basal metabolism determinations were made. These show a distinct decline in the metabolic rate. The averages of these are 34.3 calories per square meter of body surface and −13.3 per cent for the basal metabolic rate. His average oxygen consumption per minute fell from 230 to 216 cc.

R. W. C. showed a steady decline in his basal metabolism throughout the entire period of training. For the five determinations just before he began to take vigorous exercise, the average was 36.7 calories per square meter and the average basal metabolic rate −7.4 per cent. For five determinations made during the first 18 days of training, the averages were 35.6 calories and −9.8 per cent for the basal rate. For five days during the middle of the training period, the averages were 34.5 calories and −13.1 per cent basal rate; and for five determinations made during the last five weeks of training, the averages were 33.3 calories and −15.4 per cent basal metabolic rate.

E. C. S. likewise showed a decline in his metabolism during the period of exercise. He is also a slender non-athletic type and is past middle life. He took vigorous exercise for an hour daily from October 17 to December 19. Our "out of training" observations on him cover a pre- and post-training period. Early in January he underwent an appendectomy, after which our first determination of his gaseous metabolism was made on February 6. In table 1 appear his averages for all determinations made while both in and out of training. His average oxygen consumption per minute when out of training was 221 cc., and when in training only 208 cc. The calories per square meter of body surface showed a similar decline, falling from an out-of-training average of 36.8 to an in-training average of 34.3. His normal basal metabolic rate was −4.9 per cent, while during the training period it averaged −10.5 per cent.

The full effect of exercise on the gaseous metabolism of E. C. S. seemed to be reached in about three weeks. The metabolic rate at the end of the first week of training was −7.5 per cent; at the end of the second week, −4.9 per cent; and at the end of the third week, −10.4 per cent. It vacillated around this value during the balance of the period of training. The calories per square meter of body surface for the first three weeks of training were 35.5, 36.5 and 34.4 respectively.

Only four determinations of the metabolism of E. C. S. were made after the training period. These were on February 6, March 19 and April 9 and 30. The calories per square meter of body surface were 36.0, 36.1, 34.6 and 37 respectively; and basal metabolic rates, −6.2, −6.2, −6.0 and −3.8 respectively. It is evident from these data that the effect of a period of two months of regular vigorous exercise on the gaseous metabolism of the body is not lasting. However, it appears that the gaseous metabolism of the body did not wholly return to the pre-exercise normal

until in April. The averages for all determinations on him before the training period were 37.7 calories and −4.3 per cent basal metabolic rate.

The observations on G. C. R. include ten determinations in the out-of-training condition, some of which were made in September and others the following January to April. There were ten observations in the period of training which extended from October 12 to December 16. The exercise taken by G. C. R. was, on the whole, more vigorous than that taken by E. C. S. and frequently was continued through two hours. The averages for all determinations which appear in table 1 show that his basal gaseous metabolism was not definitely influenced by regular physical work. His oxygen consumption per minute averaged 231 cc. when he was out of condition and 227 cc. during the training period. The average calories per square meter of body surface were 39.3 when out of training and 38.7 when in training, while the basal metabolic rate for the two conditions averaged −0.4 and −2 respectively. In the post-training period, when he took no exercise whatever and was so overworked that he was constantly fatigued, the metabolism still remained constant. The last four determinations during this period, which were made in March and April, gave an average of 38.8 calories and −1.5 per cent basal metabolic rate.

A. L. H. was another non-athlete who showed no change in the basal metabolic rate during training. We made only three determinations before he began to train and but five during a seven weeks period of training. His calories averaged 36.4 before training and 36.7 in the training period, while the average basal metabolic rate was −7.9 and −7.1 per cent during the two periods.

We had the privilege of determining the respiratory exchange of the center of our varsity football team eight times during the playing season and seven times during the following five months. This man, W. F. A. in table 1, showed an unusually high rate of metabolism, and in this he resembled the large athletes examined by Benedict and Smith (3). However, his metabolism when he was not in training was distinctly higher than during training. His total oxygen consumption per minute was not greatly different for the two conditions, the average was 293 cc. when out and 289 cc. when in training. The calories per square meter of body surface per hour averaged 45.0 when he was not training and 43.1 when he was in training. The normal, or out of training, basal metabolic rate was +14 per cent and the average for the training period only +9.5 per cent.

After the football season W. F. A. took almost no exercise. He then kept very irregular hours, seldom got to bed before twelve o'clock, and often studied all night for an examination. He continually reported that he felt flabby. These habits of work may account for the fact that, when we made our first post-season determination, ten days after he stopped training, the basal metabolic rate was already up to +15.8 per cent. It remained around that level during the following five months.

Our experiments were not planned for the purpose of comparing the athlete and non-athlete, but to find the effect of a period of training on the basal respiratory exchange when any individual indulges in regular physical exercise. Our results with the athlete suggest that the findings of Benedict and Smith may, on the whole, be correct, in that the athlete may normally have a more active basal metabolism than the non-athlete. But our results show that it is incorrect to assume therefore that training augments the basal metabolism. The actual effect of training may be quite the reverse. From our data it appears that a period of training may or may not influence the basal metabolism, and that if it does do so, there is a decline in the basal respiratory exchange.

The exercise taken by our subjects resulted in a feeling of well being and in increased alertness. It increased the appetite for food, improved the muscles, and augmented the capacity for doing physical work. Whether it increased the total muscle mass of the body we cannot say. None of the men showed a large change in weight. All of the non-athletes were of the slender type and none had much fat to lose. If there was growth in the size of the muscles during training, then we conclude, because of the failure of the basal metabolism to increase in two of the subjects, and its decline in the others, that the oxygen consumption of the muscles of the body during basal conditions is decreased. It therefore appears that one of the results of physical training is an increase in the efficiency with which basic life processes are carried on.

It is still an open question as to what causes the decline in the basal metabolism in "cage life." May it not be that other factors, such as lack of sunshine, also influence the metabolism? Lindhard (8) found that the oxygen consumption per kilogram and per hour increased with the advance of the seasons from winter to summer; while Hill, Campbell and Gauvain (7) showed that metabolism increased in children undergoing the open-air treatment.

FREQUENCY AND VOLUME OF BREATHING. The trained man is known ordinarily to breathe slower and deeper than the untrained. This applies to observations made in the laboratory, examination room, and on the field. We have not found records of observations for these factors under basal conditions.

According to our records the basal rate of breathing is not affected by training. The average frequency of breathing for our subjects, when both out of and in training, was as follows: R. W. C., 10.7 and 10.3; E. S. C., 9.6 and 9.2; A. L. H., 11 and 11; G. C. R., 16.5 and 17.9; and W. F. A., 16 and 15.5.

The basal minute-volume of breathing (see table 1) was not clearly altered. E. C. S. is the only one who showed anything like a measurable change. In the pre-training period he averaged 4.78 liters per minute;

during training, 4.60 liters; and in the post-training period, 5.30 liters. This increase may have been brought about by the appendectomy experience. It is clear that training did not decrease the volume, as the averages for the pre-training and training periods are as nearly equal as could be expected.

PULSE RATE. A decrease in the frequency of the heart beat has often been observed during physical training. Cook and Pembrey (4) have pointed out that, while there is considerable variation in the pulse rate of different healthy individuals, a slow rate is more frequent among men trained for muscular work. Dawson (5) found that the greatest retardation of the resting pulse rate occurred during noon and afternoon. He also

TABLE 2

Basal and early morning circulatory data

PERIOD	W.F.A.			G.C.R.			E.C.S.		
	Pulse rate	Arterial pressure		Pulse rate	Arterial pressure		Pulse rate	Arterial pressure	
		Systolic	Diastolic		Systolic	Diastolic		Systolic	Diastolic
Reclining posture									
		mm.	*mm.*		*mm.*	*mm.*		*mm.*	*mm.*
Pre-training				55	104	80	65	104	72
Training	48	107	76	55	106	83	62	104	67
Post-training	50	103	73	56	109	85	69	104	67
Standing posture									
Pre-training				77	117	91	75	114	83
Training	62	122	99	71	111	96	71	113	80
Post-training	65	115	91	75	121	99	84	112	79

observed that illness at least partly obscured this effect. There are no data on record regarding the continuousness of the retarding influence. Dawson's work indicates that it is less evident during the early part of the day.

A summary of our pulse rate study of three subjects is given in table 2. The reclining posture, or basal pulse rate was counted in the early morning while the subject was still in bed, perfectly quiet and relaxed; and the standing posture rate was taken two minutes after he got out of bed, as he stood at the bedside, and before any unnecessary movements had been made.

Training slightly retarded the pulse rate of the basal condition in four of our five subjects. A decided reduction would hardly be expected be-

cause of the slowness of the early morning pulse. During training the average frequency of the basal pulse rate declined from 53 to 51 in R. W. C., from 71 to 68 in A. L. H., and from 65 to 62 in E. C. S. In the post-training period, the pulse rate increased from 48 to 50 for W. F. A. and from 62 to 69 beats for E. C. S. G. C. R. showed no change either during or after training.

Of the four men whose basal pulse rate was slower during the training period, only three had also a decrease in the basal metabolic rate. It is nevertheless interesting to find the decline in pulse rate so definitely associated with that of the metabolic rate, since this is in accord with the relationship established between the pulse rate and the gaseous exchange by Harris and Benedict (6).

The pulse rate changes for the standing posture were followed in three cases. Each man had a slower pulse rate while standing when in training than when out of training. One case, G. C. R., had failed to show this influence of training when in bed, but as soon as he got out the retarding influence manifested itself. For him the average pre-training period rate was 77, during the training period it declined to 71, and in the post-training period it advanced to 75. E. C. S. showed during training a decline from an average of 75 to 71, and in the post-training period an acceleration from an average of 71 to 84. It should be noted here that E. C. S. had an attack of appendicitis during the later period. This may account for the higher post- than pre-training rate. W. F. A., the athlete, also experienced a higher standing pulse rate when out of training; from an average of 62 in training the rate advanced to 65 when he was out of training.

While the above changes in pulse rate are not large, the fact that they occurred in each of our subjects leads to the conclusion that physical training retards the pulse rate in sleep as well as during day time activities. These changes also support the conclusion that the basic processes of the body become more efficient during a period of physical training.

ARTERIAL BLOOD PRESSURE. Dawson (5) believes that training does not produce any constant changes in blood pressure. Bainbridge (1) states that "the systolic arterial pressure, according to most observers, is not higher during rest in trained than in untrained men." Our results (see table 2) agree well with this; since in three men studied training did not appear to influence, in a definite manner, either the basal or the standing blood pressures.

SUMMARY

The effects of physical training have been studied during the pre-training, training and post-training periods.

During the period of training the basal metabolism declined in three and

was unchanged in two subjects. In the post-training period it returned to the pre-training rate.

The basal minute-volume and frequency of breathing were not affected by training.

Training slowed the basal, and early morning standing posture pulse rates, but did not affect the early morning arterial blood pressure.

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INORGANIC CONSTITUENTS OF HUMAN SALIVA. II[1]

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Extensive experiments with subjects on known food intake (1) showed that the inorganic constituents of a given individual's saliva varied over a considerable range. In this same work it was also found impossible to establish any relationship between either the amount of a given element ingested or the amount retained and that appearing in the circulating blood or in the resting saliva.

Unquestionably the inorganic constituents of the saliva are derived from the blood circulating through the salivary glands, but the secretory and excretory functions of these glands are dominated by a complex nervous mechanism and the secretion and composition of the saliva may therefore be influenced by a number of uncontrollable stimuli: i.e., olfactory, gustatory, auditory, visual.

The work reported in this paper has been confined to a detailed study of the variations of certain of the inorganic constituents of the saliva. In attempting to ascertain the rôle of saliva in oral diseases it is obvious that resting saliva, because it is bathing the teeth and oral tissues a greater portion of the time, is the type of secretion that should be studied. However, since large samples of paraffin-activated saliva may be obtained in a few minutes, it seemed desirable to make simultaneous studies of the two types of secretion. It also seemed advisable to ascertain the effects of removing the suspended material which is present in practically every sample of saliva (especially in resting samples). Finally, through rather radical diets we hoped to demonstrate quantitative changes in the composition of saliva.

METHODS. *Sampling.* With the subjects in a quiet place, resting saliva was first collected and immediately following this the paraffin-acti-

[1] This work, carried out under the auspices of the original California Stomatological Research Group, was supported in part by grants from the Carnegie Corporation, the American Dental Association and the Associated Radiographic Laboratories of San Francisco.

[2] The material submitted in this paper forms part of a thesis submitted by Lena Levine in partial fulfillment of the requirements for the degree of Master of Science in the Graduate School of the University of California.

vated specimen was obtained. Before removing samples for analysis the saliva was thoroughly mixed in order to distribute any sediment (food particles, desquamated cells, etc.).

Analyses: Total solids. Ten cubic centimeters of saliva, in a weighed platinum dish, were evaporated to dryness over a steam bath, and then heated in an air oven at 100°C. for ten minutes. The residue was not allowed to remain in the oven longer than ten minutes because it was found that caramelization resulted from continued heating. It is probable that even with this precaution, a small amount of the more volatile organic matter may have been decomposed. After cooling in a desiccator the dish and contents were weighted.

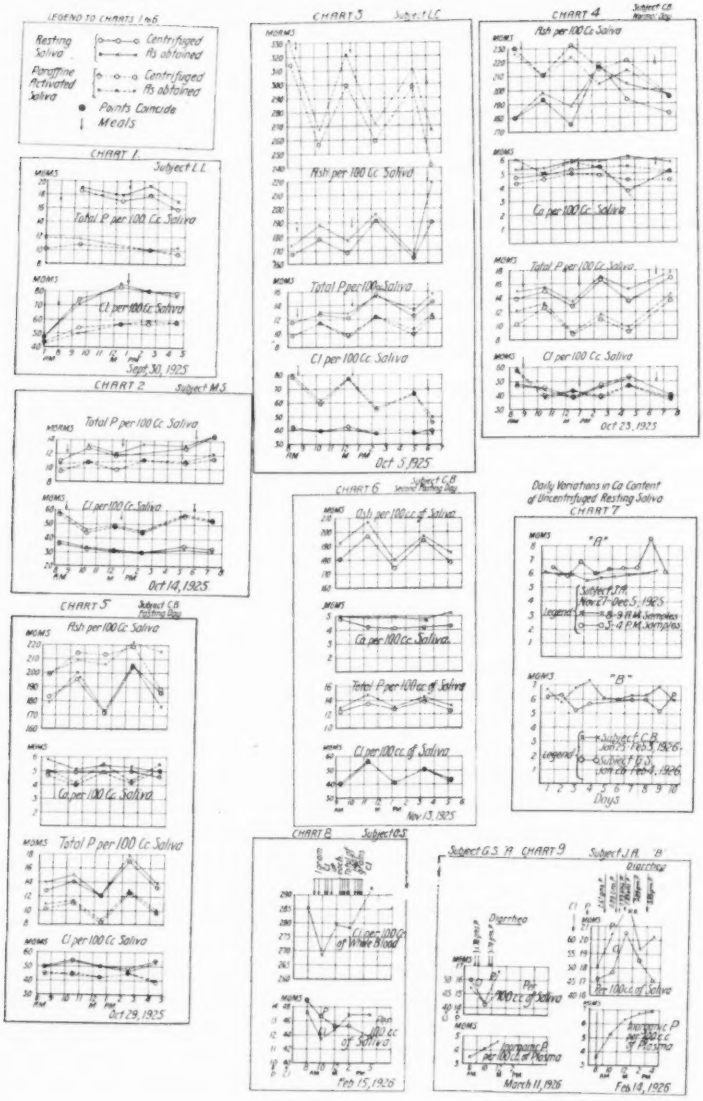
Ash. The total solids residue was heated in an electric muffle furnace at dull red heat until a white ash was obtained. After cooling in a desiccator and weighing, the ash was dissolved in approximately 5 cc. of distilled water and 0.1 cc. concentrated hydrochloric acid, digested on a steam bath, filtered if necessary, and made up to volume of 25 cc. Calcium and phosphorus determinations were subsequently made on aliquots of such solutions.

Phosphorus. Total phosphorus of the saliva (using an aliquot of the above solution) and the inorganic phosphorus in blood plasma were determined by the method of Benedict and Theis (2). The method of deproteinization used by Updegraff and Lewis (3) was not practical here because of the variable amounts of inorganic matter brought into solution. Although trichloroacetic acid removed most of the protein, the amount left gave a turbid solution with the Benedict-Theis reagents. Inorganic phosphate in urine was determined by the method of Fiske and Subbarrow (4).

Calcium. Calcium was determined by the method of Clark (5).

Chlorin. Using 1 cc. of fresh saliva for each determination the chlorids in saliva were determined by the method of Van Slyke (6). Chlorids in blood were also determined by this method. Whitehorn's method (7) was used for the determination of chlorids in urine.

EXPERIMENTAL. During the hour or more necessary for the collection of 50 cc. of resting saliva considerable material is deposited in the collecting vessel. It was not known whether these deposits resulted from the precipitation of certain substances (calcium, phosphorus, mucin) or were merely due to the sedimentation of desquamated cells, food particles, bacteria, etc., or to both. In order to study this point large amounts (50 cc.) of resting or paraffin-activated saliva were collected from several subjects. One-half of each sample was immediately centrifuged at high speed for ten minutes, this being sufficient to remove the material in suspension. We have summarized the data from several subjects for chlorin, phosphorus and calcium in charts 1 to 6. These elements were selected for the following reasons: *a*, of the various constituents present in saliva,


LEGEND TO CHARTS 1 to 6
Resting Saliva
Centrifuged
Its obtained
Paraffine Activated Saliva
Centrifuged
Its obtained
Points Coincide
Meals
CHART 1
Subject L.L.
Total P per 100 Cc. Saliva
Cl per 100 Cc. Saliva
Sept. 30, 1925
CHART 2
Subject M.S.
Total P per 100 Cc. Saliva
Cl per 100 Cc. Saliva
Oct 14, 1925
CHART 3
Subject L.C.
Ash per 100 Cc. Saliva
Total P per 100 Cc. Saliva
Cl per 100 Cc. Saliva
Oct 5, 1925
CHART 4
Subject C.B.
Normal Day
Ash per 100 Cc. Saliva
Ca per 100 Cc. Saliva
Total P per 100 Cc. Saliva
Cl per 100 Cc. Saliva
Oct 23, 1925
CHART 5
Subject C.B.
Fasting Day
Ash per 100 Cc. Saliva
Ca per 100 Cc. Saliva
Total P per 100 Cc. Saliva
Cl per 100 Cc. Saliva
Oct 29, 1925
CHART 6
Subject C.B.
Normal Fasting Day
Ash per 100 cc. of Saliva
Ca per 100 cc. Saliva.
Total P per 100 cc. of Saliva
Cl per 100 cc. of Saliva
Nov 13, 1925
Daily Variations in Ca Content of Uncentrifuged Resting Saliva
CHART 7
"A"
Subject J.A.
Nov 27-Dec 5, 1925
Legend
8-9 A.M. Samples
5-6 P.M. samples
"B"
Subject C.B.
Jan 25-Feb 3, 1926.
Subject J.A.
Legend
Jan 28-Feb 4, 1926
Days
CHART 8
Subject G.S.
Cl per 100 Cc of Whole Blood
Ash per 100 cc of Saliva
Feb 15, 1926
Subject G.S. 'A'
CHART 9
Subject J.A. 'B'
Diarrhea
Diarrhea
Ash per 100 cc of Saliva
Inorganic P per 100 cc of Plasma
March 11, 1926
Diarrhea
Ca per 100 cc of Saliva
Inorganic P per 100 cc of Plasma
Feb 14, 1926

they can be determined with the greatest accuracy; *b*, the chlorides being highly soluble, should not be affected either by precipitation or sedimentation and variations in the chlorid content of the uncentrifuged and centrifuged saliva should indicate the magnitude of the experimental errors; *c*, if due to changes in reaction,[3] the deposits would then be mainly the result of precipitation and one should expect the phosphorus and calcium to be removed in a definite ratio: i.e., 3 Ca: 2 P in case of $Ca_3(PO_4)_2$ Ca: P if $CaHPO_4$ were formed.

TABLE 1

*Effects of centrifuging upon the Cl, P and Ca content of saliva**

DATE	SUBJECTS	NUMBER OF ANALYSES	RESTING SALIVA			PARAFFIN-ACTIVATED SALIVA		
			Cl	P	Ca	Cl	P	Ca
1925			*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
October 5	L. C.	6	101	94		98	96	
October 14	M. S.	6	101	94		98	95	
October 23	C. B.	6	102	95	84	102	93	82
October 29	C. B.†	5	103	94	91	100	97	92
November 13	C. B.†	5	101	95	87			
November 27 to December 5	J. A.‡	9	99§	90§				
		9	100‖	90‖				
1926								
January 26 to February 4	G. S.‡	8	98	93	94			
January 25 to February 3	C. B.‡	10	100	91				

* In making up this table the average value of each element in the centrifuged sample is divided by the average value of the element in the corresponding uncentrifuged sample and the results expressed in per cent (taking the uncentrifuged value as 100 per cent).

† Subject C. B. fasted on both days.

‡ Daily determination. Preceding figures are for several determinations made during the same day.

§ A.M. samples.

‖ P.M. samples.

While paraffin-activated and resting saliva are quantitatively very different in composition,[4] it can be seen that the variations in the chlorin, phosphorus and calcium content are very similar. A brief account of approximately 90 determinations each of chlorids and phosphorus and

[3] Hall and Westbay (8) and Clark and Carter (9) found no significant changes in the reaction of saliva over a period of 3 to 5 hours.

[4] Smith (10) noted a very marked increase in the bicarbonate content of saliva obtained by paraffin activation. Chittenden and Richards (11), McClelland (12) and Starr (13) found that mastication, regardless of what is chewed, increased the pH.

35 calcium determinations (5 subjects) is presented in table 1. The results given in charts 1 to 6 and in table 1 show that the chlorid content of both resting and paraffin-activated saliva was not affected by the removal of the suspended material. The maximum deviation observed was 3 per cent (subject C. B., October 29) which is not beyond the limits of experimental error. Concerning the chlorid content of paraffin-activated saliva, it was interesting to find that seventeen experimental subjects divided themselves into three distinct groups: a, four in which the chlorid content of the two types of saliva was practically the same; b, five in which the chlorids were highest in the resting saliva, and c, eight in which the chlorids were highest in the paraffin activated samples. In three of the latter subjects the chlorid content of the paraffin-activated saliva was 2 to 3 times that of the resting sample.[5] In chart 3 attention is called to the unusually uniform chlorid content of the resting saliva and the extreme variability in the paraffin-activated samples.

The phosphorus content of the centrifuged samples of both resting and paraffin-activated saliva was always lower than that of the corresponding uncentrifuged samples. In 28 determinations (3 subjects) the loss did not exceed 6 per cent but in 36 determinations (3 subjects) the loss was 7 to 10 per cent (table 1), which is more than should be attributed to experimental errors.[6]

The calcium was consistently lower in the centrifuged samples of both types of saliva, the loss varying from 6 to 18 per cent and only once (subject G. S.) approximated the experimental error of the method.[7] The work of Holt, Chown and La Mer (14) indicates that the precipitation of $Ca_3(PO_4)_2$ from biological fluids, supersaturated with respect to this compound, is a very slow process, and also that the solubility of $Ca_3(PO_4)_2$ is greatly altered by various neutral salts. R. N. Loomis, working in this laboratory was not able to prepare mucin free from calcium and phosphorus.[8] He also found that large amounts of calcium were removed from

[5] Repeated determinations, made several weeks apart, gave the same general results, i.e.:

G.C.–9–10–25	Resting saliva	53 mgm. Cl per 100 cc.
	Paraffin activated saliva	116 mgm. Cl per 100 cc.
G.C.–3–10–26	Resting saliva	76 mgm. Cl per 100 cc.
	Paraffin activated saliva	123 mgm. Cl per 100 cc.

[6] It is possible that the decreased phosphorus content of the centrifuged saliva may be partially due to the removal of cellular material rich in phosphorus; i.e., the desquamated epithelial cells of the buccal cavity. However, the fact that all of the phosphorus in the saliva is present in the inorganic form (1) is strong evidence against this idea.

[7] A number of investigators have shown that it is possible to make micro determinations of calcium with an accuracy of 3 to 5 per cent. We are indebted to Dr. H. Updegraff for the calcium determinations reported in this paper.

[8] Personal communication from R. N. Loomis.

solution when the pH was favorable for the precipitation of mucin. Our work fails to show the exact mechanism by which calcium and phosphorus are removed from solution during the time of collection and centrifugalization. However, from evidence in hand it seems unlikely that the calcium and phosphorus are precipitated either as $Ca_3(PO_4)_2$ or as $CaHPO_4$ and there is a strong suggestion that these elements may be removed in the form of a Ca-P-mucin complex.

Although Chittenden and Richards (11) found that the ingestion of food did not appreciably alter the composition of the saliva, we obtained certain rhythmical variations in the different constituents which seemed to be related to the ingestion of food (see charts 1, 2 and 3). The periodic variations were especially noticeable in the ash and phosphorus content. While studying the neutralizing power (buffer content) of saliva, Marshall (15) found that decreases in the ash content were accompanied by a lowered neutralizing power. While he did not actually determine the amounts of the different buffering substances, it is obvious that the inorganic phosphates and bicarbonates would be involved in such a relationship. The data in charts 3, 4, 5 and 6 show that in many instances the phosphorus and ash of both types of saliva (uncentrifuged and centrifuged) varied in the same direction; i.e., a high ash with a high phosphorus and a low ash with a low phosphorus. Variations in the ash and chlorid content of paraffin-activated saliva commonly paralleled each other, note especially subject L.C., chart 3. From the data presented in table 2, it is evident that there are many exceptions; i.e., between 8 and 10 a.m., subject L.C. showed a 20 per cent decrease in the ash accompanied by a 30 per cent increase in the phosphorus, and between 10 a.m. and 12 m. a 20 per cent increase in ash was accompanied by a 25 per cent increase in phosphorus. Similarly, subject M. S. showed an increase of 13 per cent in the ash content with no change in the phosphorus, and subject C. B. a 5 per cent increase in ash with a 20 per cent increase in phosphorus. In order to further test whether the ingestion of food had any effect on the character of the periodic variations, subject C. B. fasted on two different occasions, the results of which are given in charts 5 and 6. As can be seen the variations in the ash, chlorin and phosphorus content are as extreme and similar to those obtained with this subject under normal food intake, chart 4. In an attempt to establish more points on the periodic curves we took saliva samples an hour later during a second fasting experiment. On the first fasting day the low values for ash and phosphorus were obtained at 12 m. and again at 4:30 p.m. On the second day, under the same conditions, similar drops in ash, phosphorus and chlorin were obtained at 1:15 p.m. When food was ingested the low points for phosphorus came at 12:30 p.m. and 4:30 p.m.; the ash, however, fell steadily from 2:30 to 7:30 p.m. While we have no quantitative data on the food eaten, the general qualitative differences in

TABLE 2

The effect of diet upon the composition of saliva

TIME	Ash		P		Cl		
	Resting	Paraffin	Resting	Paraffin	Resting	Paraffin	
			Subject L. C., October 5, 1925				
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
8:15 a.m.	174	332	12	10	40	80	
8:40 a.m.	Breakfast.						Egg, toast, jam, milk
10:25 a.m.	188	268	13	12	40	62	
12:15 m.	177	321	13	9	40	77	
12:35 m.	Lunch.						Egg salad, bread, cake
2:15 p.m.	196	271	15	12	38	56	
5:00 p.m.	167	310	13	11	38	67	
	Dinner.						Lamb chop, vegetables, bread milk
7:15 p.m.	219	266	16	13	38	50	
			Subject L. C., November 10, 1925				
7:40 a.m.	...	315	..	11	..	71	
8:00 a.m.	Breakfast.						Orange, bacon, egg, toast, jam, milk
10:10 a.m.	...	252	..	12	..	53	
12:10 m.	...	262	..	11	..	59	
12:15 m	Lunch.						Apple, milk, cake, candy
2:10 p.m.	...	260	..	11	..	55	
5:05 p.m.	...	244	..	10	..	55	
6:30 p.m.	Dinner.						Vegetable-nut salad, roast beef, vegetables, milk, pudding
7:40 p.m.	...	220	..	13	..	49	
			Subject M. S., October 14, 1925				
8:20 a.m.	158	239	11	11	38	58	
8:50 a.m.	Breakfast.						Toast, ham, potatoes, coffee
10:25 a.m.	172	224	13	11	33	46	
12:10 m.	170	241	12	10	32	49	
12:45 m.	Lunch.						3 sandwiches, doughnut, grapes
2:30 p.m.	182	213	14	11	30	45	
5:20 p.m.	179	244	13	11	33	57	
	Dinner.						Soup, crackers, bread pudding, milk
7:25 p.m.	168	222	15	12	31	54	

TABLE 2—*Concluded*

TIME	Ash		P		Cl		
	Resting	Paraffin	Resting	Paraffin	Resting	Paraffin	
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
Subject C. B., October 23, 1925							
8:25 a.m.	180	226	15	12	48	58	
9:00 a.m.	Breakfast...						Toast, milk
10:25 a.m.	198	210	16	13	44	38	
12:25 m.	188	223	13	9	38	43	
1:00 p.m.	Lunch.....						Ham sandwich, milk
2:30 p.m.	217	204	17	12	46	38	
5:20 p.m.	204	214	15	10	52	47	
6:30 p.m.	Dinner.....						Veal chops, fried potatoes,
7:30 p.m.	197	195	18	15	40	37	bread
Subject C. B., October 29, 1925—fasting day							
8:35 a.m.	179	200	14	11	50	50	
10:25 a.m.	200	209	15	11	53	54	
12:25 m.	173	206	13	9	50	51	
2:25 p.m.	206	222	17	13	47	49	
4:40 p.m.	186	205	14	10	50	53	

the diets of the three subjects together with the results of the two fasting days show quite conclusively that the composition of saliva is not materially changed by the ingestion of various types of food. It is well known that abnormal amounts of fat, sugar, etc., retard gastric secretion and digestion and it is quite conceivable that the stimulation of the salivary glands by sugar may result in a temporary suppression of their activity. The saliva immediately obtained may therefore show some significant changes as has been reported by Pickerill (16) and Marshall (15). However, these changes are transitory and there is little doubt that the saliva contains at all times an excess of buffering substances. This, together with the constant supply of fresh secretion provides the buccal cavity, as a whole, with a very efficient protective mechanism against the injurious action of acids formed either in the processes of digestion or through bacterial activity. It is obvious that this statement would not include pyorrhea pockets, plaques, etc.

Finding that the calcium did not follow the periodic variations observed for the ash and phosphorus we were led to make a more detailed study of this element. Subject J. A. collected resting saliva between 8 and 9 a.m. and again between 3 and 4 p.m. for 9 successive days. The results, given in chart 7A show very uniform calcium values in the morning samples but as much as 32 per cent variation between the highest and lowest values

of the afternoon samples. Subjects C. B. and G. S. collected daily samples of resting saliva on 10 successive days. As can be seen from the data in chart 7B both subjects showed considerable variation, 21 per cent difference between highest and lowest values for subject C. B. and 19 per cent for subject G. S. This work is in agreement with that reported in an earlier paper (1) and also with that of Spencer-Payne (17) who found that the calcium content of the saliva varied from hour to hour and day to day. Only on the second fasting day, subject C. B., chart 3, and in the morning samples of subject J. A., chart 7A, did we find any resemblance of a uniform calcium content. It is very difficult to see how Bunting and Rickert (18) were able to predict the calcium content of the saliva from the condition of the teeth and also how Ferris (19) could predict from an analysis of the saliva of a nursing mother whether or not her milk would be suitable food for her child.

Although individual determinations varied as much as 40 per cent,[9] the calcium was, generally speaking, the most constant of the constituents studied.

Having been unable to show any constancy in the composition of saliva under regulated dietary conditions (1), and finding as great variation under fasting conditions as when food was ingested, it seemed inadvisable to attempt further studies with diets as we had originally planned to do.

Knowing that the ingestion of inorganic phosphate is followed by a rapid excretion of phosphate in the saliva and having found such striking increases in the chlorid content of the paraffin activated saliva, we decided to make post-absorptive studies of the chlorid and phosphorus content of blood and resting saliva after oral ingesiion of relatively large amounts of these substances.

Subject G. S. drank 1400 cc. of a chlorid solution[10] equivalent to 14.07 grams of Cl, between 8:55 a.m. and 3:25 p.m. The results of the experiment are shown in chart 8. Following the ingestion of 200 cc. of solution (2.0 grams Cl) there was a decrease of 6 per cent in the chlorid content of the whole blood and a simultaneous decrease of 8 per cent in the chlorid content of the saliva. Concurrent with these changes there was also a decrease of 8 per cent in the phosphorus content of the saliva. Subsequently the chlorid content of both blood and saliva increased, so that at 5 p.m. the saliva and blood had returned to practically normal values. The phosphorus content of the saliva, however, continued to decline, the last determination being 18 per cent below the normal. There was no indication of diuresis and only 62 per cent of the ingested chlorids appeared in the urine during the 12 hour experimental period.

[9] Subject J. A., December 4, a.m. sample 6.0; p.m. sample 8.5 mgm. Ca per 100 cc. of saliva.

[10] This solution contained 10 grams NaCl, 5 grams KCl and 3.3 grams $CaCl_2 \cdot 2H_2O$, equivalent to 10.0 grams of Cl per liter.

Similar experiments were carried out in which large amounts of inorganic phosphate were ingested orally. Between 8 a.m. and 12 m. subject J. A. drank 600 cc. of phosphate solution,[11] equivalent to 5.34 grams of P (see chart 9A). During this time there was an 80 per cent increase in the phosphorus content of the saliva and concurrently an increase of 75 per cent in the inorganic phosphorus of the blood plasma. Rather severe diarrhea developed at this time with the result that the concentration of phosphorus in the saliva fell very rapidly (59 per cent in 2 hours). The inorganic phosphorus of the plasma increased slightly during the remainder of the day, indicating absorption of a small amount of the 1.78 grams of P ingested after 12 m. A second experiment with the phosphate solution, subject G. S., chart 9B, was less satisfactory because severe diarrhea ensued, after the ingestion of only 400 cc. of the phosphate solution (3.56 grams of P). Similar to the experiment with the chlorid solution (chart 8) this subject showed a marked preliminary drop (17 per cent) in the phosphorus content of the saliva, followed by a return to slightly above normal. During the same time there was a steady increase (31 per cent) in the inorganic phosphorus of the plasma.[12]

Although there were no chlorids in the phosphate solution, the chlorid content of the saliva of subject J. A. increased approximately 35 per cent in 4 hours. As can be seen from chart 9A the trend of the curves for phosphorus and chlorids in the saliva is very similar. The variations in the chlorid content of the saliva of subject G. S., chart 9B, were similar to those of the phosphorus and in the experiment with the chlorid solution it can be seen (chart 8) that the phosphorus content of the saliva changed in a similar way to that of the chlorids. We have no explanation to account for this apparent Cl-P relationship, in case of phosphate the salivary glands function very distinctly as excretory organs for as soon as the colon became active in eliminating this foreign substance there was a very rapid decrease in the phosphorus of the saliva. When subject G. S. ingested 14.07 grams of Cl (equivalent to 23 grams of NaCl) under post-absorptive conditions and when only 67 per cent was excreted in the urine during the 12 hour experimental period, it is difficult to account for the slight variations found in the chlorid content of the resting saliva.

Bunting and Wixon (21) fed large oral doses (about 3 grams per day) of calcium chlorid and lactate, both with and without phosphorus, to human subjects but were unable to demonstrate any increase in the calcium content of the saliva. They also found a normal calcium content in the saliva of patients showing excessive deposits and formation of "tartar."

[11] This solution contained 91 grams $Na_2HPO_4 12H_2O$ and 6.4 grams $NaH_2PO_4 H_2O$, equivalent to 8.89 grams of P per liter.

[12] Following intravenous injections of phosphate solutions Barkus (20) found a marked retention of inorganic phosphate in the plasma.

It should also be mentioned that attempts to increase the calcium content of the blood through repeated oral and hypodermic administrations of calcium salts have not been successful (22) (23) (24) (25).

In addition to the work reported we also determined the total solids, organic matter and ash in all samples, but it has not seemed advisable to include the results in this discussion.

SUMMARY

The work reported in this paper indicates that:

1. The inorganic constituents of paraffin activated saliva show greater quantitative variations than those of resting saliva.

2. Removal of desquamated cells, food particles, etc., by centrifuging results in a loss of both calcium and phosphorus. It is suggested that these elements are removed in the form of a Ca-P-mucin complex rather than as $Ca_3(PO_4)_2$ or $CaHPO_4$.

3. It is not possible to appreciably change the composition of the saliva by short fasting.

4. It is difficult to increase the chlorid content of resting saliva by the oral ingestion of large amounts of soluble chlorids.

5. With respect to abnormal ingestion of inorganic phosphorus the salivary glands exhibit a marked excretory function.

6. Variations of the several constituents of the saliva may be the result of physiological rhythm.

In conclusion it may be said that each individual reacts differently to stimulation and the individual response to the same type of stimulation varies from time to time with the result that the normal variations in the content of the respective inorganic constituents are sufficiently large to disguise any relationship that may exist. Our results are therefore in accord with the earlier work of Chittenden and Richards (11), Lothrop and Gies (26), Spencer-Payne (15), and with that reported in a previous paper.

Our thanks are due to the various persons who so kindly coöperated in these experiments.

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THE EFFECT OF ADRENALIN ON PHOSPHORUS PARTITION IN MUSCLE

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In one of the catabolic phases of carbohydrate metabolism, namely, the performance of muscular work, the rôle of "lactacidogen," a hexose-diphosphoric acid discovered in striated muscle by Embden (1921) and his collaborators, has aroused considerable interest. One line of attack on the problem is to study the effect of physiological substances involved in carbohydrate metabolism, namely, insulin and adrenalin. There has been some work on the effect of insulin on lactacidogen (Audova and Wagner, 1924; Eadie, Macleod and Noble, 1925; Collazo, Händel and Rubino, 1924; Kay and Robinson, 1924; Sokhey and Allen, 1924; Harrop and Benedict, 1925), but the data do not permit the drawing of any conclusion. The present investigation was undertaken to determine the effect of adrenalin on lactacidogen.

Beattie and Milroy (1925) have reported experiments with adrenalin on two cats, killed three hours after the injection of adrenalin. They observed a slight decrease in the lactacidogen content of the muscle. Their figures on one of the cats fail to show any effect of the adrenalin on the phosphorus partition between inorganic and lactacidogen phosphorus.

The method used in this work was the following (rabbits were used throughout): The animal was killed by the injection of 2 cc. of chloroform into the heart. The white muscle of the thigh was then excised as rapidly as possible and run through a cold meat chopper. One portion of the hashed muscle was immediately placed in a museum jar of about 200 cc. capacity, containing about 75 cc. of 5 per cent trichloracetic acid, which had been weighed previously. Roughly, 10 grams of hashed muscle were added and the contents well shaken to precipitate proteins and stop autolysis. Another portion of the muscle hash was placed in a similar jar containing 25 cc. of 3 per cent sodium bicarbonate solution. Both jars were weighed, thus giving the weight of muscle by difference. The jar containing the muscle and sodium bicarbonate was then put in a 37°C. incubator for three hours. Embden (1921) has shown that this treatment hydrolyses the lactacidogen completely, with the liberation of an equivalent amount of phosphoric acid.

The time elapsed from the injection of the chloroform to the addition of the hashed muscle to the contents of the museum jar was generally under three minutes and never more than five.

At the end of the three-hour period, water was added to the second jar, then 50 cc. of 10 per cent trichloracetic acid; the contents of both jars made up to volume, mixed well, and filtered. Phosphorus was determined in the filtrates by the method of Roe, Irish and Boyd (1926). Using this technique, consistent results were obtained on normal animals.

TABLE 1

Phosphorus partition in white muscle of rabbit

Milligrams of phosphorus per 100 grams muscle

RABBIT NUMBER	A. INORGANIC	B. INORGANIC PLUS LACTACIDOGEN	L. B-A LACTACIDOGEN	LACTACIDOGEN AS PER CENT OF TOTAL INORGANIC PLUS LACTACIDOGEN	REMARKS
1	89.5	150.0	60.5	40.3	Fed animal
2	83.5	141.0	57.5	40.8	Fed animal
4	84.0	129.0	43.0	33.3	Fed animal
16	92.0	132.0	40.0	30.3	Fed animal
17	88.0	157.0	69.0	44.0	Fed animal
18	91.0	139.5	48.5	34.8	Fed animal
25	83.0	129.5	46.5	35.9	24 hours without food
26	85.0	156.0	71.0	45.5	24 hours without food
27	83.0	153.0	70.0	45.8	24 hours without food
12	119.0	182.0	63.0	34.6	4 days without food
13	111.0	170.0	59.0	34.7	3 days without food
14	113.0	174.0	61.0	34.4	3 days without food
22	59.0	148.5	89.5	60.0	Fed animals
23	59.0	142.0	83.0	58.3	Kept in small cages about
24	53.0	156.0	103.0	67.3	10 weeks
29	70.5	144.0	73.5	51.0	

To study the effect of adrenalin, the following procedure was used: A blood sample was obtained by cardiac puncture. Then 0.5 mgm. per kilo of adrenalin was injected subcutaneously, followed by similar injections one and one-half and three hours after the first one. One hour after the last injection, another blood sample was obtained by cardiac puncture, the animal killed with chloroform, and the muscle worked up as stated above. Sugar was determined on the blood samples by the Folin-Wu method.

The following table shows the values for preëxisting inorganic phos-

phorus, *A*, the values after three hours' autolysis in the presence of sodium bicarbonate, *B*, and, by difference, the lactacidogen phosphorus.

It will be noted that there is no difference between fed animals and those without food for 24 hours. The slightly higher ratio of lactacidogen phosphorus to total inorganic plus lactacidogen phosphorus in these animals is probably due to their lesser muscular activity. These animals were kept in small cages which allowed little movement, while the fed ones were kept in a pen in which they could run about freely. Rabbits 22 to 24 and 29, which were kept in small cages for about ten weeks, show a marked synthesis of lactacidogen from the inorganic phosphates normally present.

The three- and four-day starved animals show a higher level of both preëxisting inorganic and total inorganic plus lactacidogen phosphorus

TABLE 2

Phosphorus partition in white muscle of rabbit following the injection of adrenalin

Inorganic phosphorus, milligrams per 100 grams muscle

RABBIT NUMBER	BLOOD SUGAR IN PER CENT NORMAL	1 HOUR AFTER LAST INJECTION	A. INORGANIC	B. INORGANIC PLUS LACTACIDOGEN	B-A LACTACIDOGEN	LACTACIDOGEN AS PER CENT OF TOTAL INORGANIC PLUS LACTACIDOGEN	REMARKS
6	0.12	0.20	135.0	149	14.0	9.0	Fed
8	0.073	1.00	142.0	182	40.0	22.0	Fed
9	0.095	1.06	135.0	157	22.0	14.0	Fed
20			139.5	165	25.5	15.0	3-day starved
21			140.0	159	19.0	13.5	3-day starved
32	0.089	0.283	120.0	153	33.0	21.6	3-day starved; one injection of adrenalin

than the fed ones, but the partition is the same as in the fed animals. The higher absolute values are probably due to dehydration incident to the starvation.

The injection of adrenalin subcutaneously into rabbits in the way stated above, was found to produce a marked increase in the preëxisting inorganic phosphorus (A values) with little or no change in the inorganic plus lactacidogen phosphorus (B values). The adrenalin has therefore produced a marked hydrolysis of the lactacidogen. In the three fed animals in the table below, the absolute lactacidogen content is less than 50 per cent of that of normal, fed animals. In one case, rabbit 6, the absolute lactacidogen content is less than 20 per cent of that of the normal animal.

This hydrolysis of lactacidogen under the influence of adrenalin is independent of the carbohydrate reserve of the animal, as it occurs in fasting as well as fed animals.

A single injection of adrenalin does not produce as marked a hydrolysis of lactacidogen as do three doses. Rabbit 32 (table 2) indicates this.

It was noted that in all the adrenalinized animals, the onset of rigor mortis was quite rapid. In the case of rabbit 9, the time for onset of rigor was about five minutes. The others did not go into rigor quite so rapidly, but the average time of onset was 15 minutes, as compared with the ordinary two hours.

SUMMARY AND CONCLUSIONS

1. Enforced inactivity results in a synthesis of the inorganic phosphates normally present in striated muscle into lactacidogen.

2. Adrenalin produces a marked hydrolysis of lactacidogen, with the formation of an equivalent amount of phosphoric acid.

3. This action of adrenalin is independent of the carbohydrate reserve of the animal.

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EFFECT OF ADRENALIN ON THE TEMPERATURE OF SKELETAL MUSCLE AFTER STOPPING THE VENOUS BLOOD FLOW THROUGH THE LIVER AND AFTER STOPPING BOTH VENOUS AND ARTERIAL BLOOD FLOW THROUGH THE LIVER

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It has been shown (Caskey, 1927) that ligation of the hepatic artery and the portal vein of dogs prevents the increase in temperature of skeletal muscle which normally follows the intravenous injection of adrenalin. Some evidence was presented to show that the loss of liver function and not operative shock was responsible for the phenomenon. The present experiments yield further evidence on this point. By stopping first venous and then arterial blood flow through the liver, it was possible to study the question of traumatic shock and also to get some idea of which part of the hepatic blood supply is more important in the response of the animal to adrenalin.

METHODS. Eight dogs were used in these experiments. The same general methods and technique were employed as were outlined by Caskey and Spencer (1926) and Caskey (1927). Fluctuations in the temperature of skeletal muscle were observed by means of a thermocouple, one junction of which was placed in a constant temperature chamber, and the other between the pectoralis major and pectoralis minor or beneath the vastus medialis muscle. Blood-pressure records were taken in some of the experiments but since they were similar in every way to those of the preceding investigations, it was regarded as an unnecessary routine procedure.

The mesenteric arteries and veins and all abdominal veins which might carry blood to the portal vein were ligated. Adrenalin was injected in pressor doses. The temperature changes in the muscle were recorded. The hepatic artery was then ligated. Adrenalin was injected and the muscle temperature changes were again observed.

OBSERVATIONS. Table 1 summarizes the principal results of these experiments. The figures show the relationships between muscle temperature and blood pressure. The data show that stopping the venous blood flow through the liver does not prevent muscle temperature from increasing as it normally does after the intravenous injection of adrenalin. In those

determinations in which blood pressure was recorded, it was found that when the venous blood flow through the liver was stopped, the blood pressure of the animal fell 60 mm. Hg.

In the second stage of the experiment the entire hepatic circulation was stopped and then the muscle temperature did not rise following the intra-

TABLE 1

Showing the effect of the intravenous injection of adrenalin on muscle temperature after stopping the venous blood flow through the liver and after stopping both venous and arterial blood flow through the liver

EXPERIMENT NUMBER		WEIGHT OF DOG	SEX	ANESTHETIC	DOSAGE OF ADRENALIN PER KILOGRAM BODY WEIGHT	TEMPERATURE IN MUSCLE AT BEGINNING OF EACH ADRENALIN INJECTION	MAXIMAL RISE IN TEMPERATURE OF MUSCLE ABOVE INITIAL TEMPERATURE	TIME INTERVAL BETWEEN INJECTION OF ADRENALIN AND MAXIMAL TEMPERATURE OF MUSCLE
		kgm.			*mgm.*	°C.	°C.	°C.
1	A*	8	F.	Barbital 30.4 grains	0.0625	37.06	0.27	3′
	B*				0.0625	37.39	0.22	4′
	C†				0.0625	37.17	(Decrease)	
	D†				0.0625	36.26	(Decrease)	
2	A*	22.25	M.	Barbital 84.55 grains	0.0337	38.87	0.11	7′
	B*				0.0337	38.90	0.07	7′30″
	C†				0.0337	38.88	(Decrease)	
	D†				0.0337	38.55	(Decrease)	
3	A*	11.65	F.	Barbital 44.27 grains	0.0429	38.12	0.07	3′30″
	B*				0.0429	38.07	0.06	4′
	C†				0.0429	37.98	(Decrease)	
4	A*	13.3	M.	Barbital 50.54 grains	0.0376	37.57	0.12	4′
	B*				0.0376	37.46	0.15	4′ 30″
	C†				0.0376	37.31	(Decrease)	
5	A*	6.8	F.	Barbital 25.84 grains	0.0736	37.80	0.05	3′
	B†				0.0736	37.73	(Decrease)	

* Indicates that venous blood flow through the liver has been stopped.

† Indicates that both venous and arterial blood flow through the liver has been stopped.

venous injection of adrenalin. However, there was a marked adrenalin pressor response and this, in spite of the further precipitous fall in the general blood pressure level of the animal.

After all circulation to the liver was stopped, muscle temperature fell at an average rate of 0.025°C. per minute. In order to determine whether

this fall in temperature was concealing tendencies to rise due to adrenalin injection, an average was taken of the rate of fall per minute during the first and second five minutes after adrenalin was given. It was found that during the first five minutes after adrenalin injection the average fall in muscle temperature was equal to 0.026°C. per minute. During the second five minutes the fall was equal to 0.024°C. per minute.

In observations on three animals in which the hepatic artery and the portal vein were both ligated at the beginning of the experiment, it was found that the average difference in fall per minute of muscle temperature

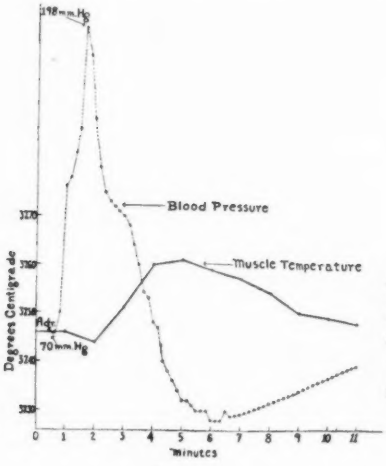

Fig. 1

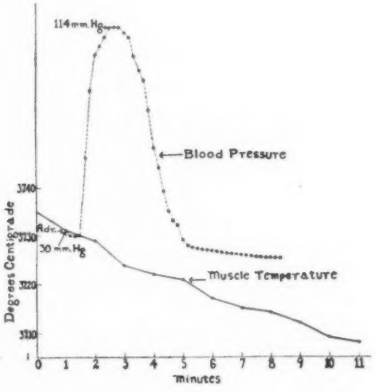

Fig. 2

Fig. 1. Blood pressure and muscle temperature after stopping the venous blood flow through the liver. *Adr.* and accompanying arrow denote adrenalin injected.

Fig. 2. Blood pressure and muscle temperature after stopping both venous and arterial blood flow through the liver. *Adr.* and accompanying arrow denote adrenalin injected.

during the first five minutes and the second five minutes of ten minute periods was equal to 0.003°C. In these three experiments there were nineteen ten minute periods. In two of these periods adrenalin was given. The average fall per minute in muscle temperature for the second five minutes of these two adrenalin periods was 0.05°C. as compared with 0.04°C., the average fall per minute for the second five minutes of the periods in which adrenalin was not given.

Discussion. General circulatory deficiency in the hepatectomized animal does not seem to be the cause of the failure of its muscle temperature to rise subsequent to the injection of adrenalin. In the hepatectomized

animal, the pressor response to adrenalin was comparable in every way to that observed in the normal animal and to that observed in the animal in which only the venous blood supply to the liver was stopped. In these experiments it was noted that the fall in blood pressure following venous exclusion was equal to or greater than that observed by Caskey after stopping the entire hepatic circulation. If fall in blood pressure were the causative factor, there would have been no adrenalin temperature response following the first stage of liver exclusion. But an increase in muscle temperature did occur, and it was comparable in height and time relationships to those rises observed in the normal animal.

The almost constant rate of fall in muscle temperature observed after total liver exclusion precludes the possibility of any adrenalin response having been concealed by the falling of the muscle temperature. This has been discussed by one of us (Caskey, 1927).

If respiration, heart rate and blood pressure are any index to systemic disturbances and traumatic shock, then certainly there was quite as much, if not more, disturbance in the first step of the two-stage liver exclusion as there was incident to the simultaneous ligation of the hepatic artery and the portal vein.

Lamson, working on the rôle of the liver in acute polycythemia, found that stopping the venous blood flow through the liver and removing the stomach, intestine, mesentery, omentum, pancreas and spleen, did not interfere with the polycythemia which follows the intravenous injection of adrenalin. It would seem in the present experiments, as in his, that the venous blood supply is not an important factor in the response of the liver to adrenalin.

The above data and their relationships as pointed out are offered as further evidence that loss of the liver as a functional unit of the body, and not simply the traumatic shock incident to its removal, is the principal factor in preventing the rise in muscle temperature in the hepatectomized animal into which adrenalin is subsequently injected.

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AN INVESTIGATION OF THE EFFECT UPON RATS OF LONG-CONTINUED INGESTION OF ZINC COMPOUNDS, WITH ESPECIAL REFERENCE TO THE RELATION OF ZINC EXCRETION TO ZINC INTAKE

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In order to obtain more precise and accurate information than now exists in the literature, upon the absorption, storage and excretion of zinc, and upon the physiological and pathological action of zinc and its compounds upon the animal body, the authors have administered daily by mouth to rats, cats and dogs varying doses of non-corrosive zinc compounds for long periods of time, and have made, during the course of the experiments, clinical and laboratory studies, which have included observations upon general health (weight determinations, appetite, nervous and digestive symptoms, etc.) and upon fertility; hemoglobin and blood cell determinations; urine findings; exact determinations of zinc intake and zinc excretion; and, finally, gross and microscopic examination of all tissues and organs following autopsy, with determinations of their zinc content. The results of these procedures with cats and dogs, together with a review of the literature upon zinc absorption, transportation, storage and excretion, and upon the effects of zinc oxide feeding have already been published (Drinker, Thompson and Marsh, 1927), and a report has been made as to the negative effect of zinc feeding upon the fertility of rats (Thompson, Marsh and Drinker, 1927).

In the experiments reported in this paper, which deal with the results of the feeding of zinc compounds to rats, we have been especially concerned with the exact observations upon the relation of zinc excretion to zinc intake and upon the effects of the ingestion of organic zinc compounds (acetate, citrate, malate) as compared with zinc oxide ingestion. The importance of the action of the organic zinc compounds at the present time depends upon the fact that it is, in the main, the organic rather than the inorganic compounds of zinc which are present as contaminations when acid foods or liquids are cooked or stored in zinc-lined containers.

The physiological effects following the administration of zinc compounds vary enormously depending upon the method of administration and especially upon the compound administered. The action of the heavy

metals consists of two parts, the local effects induced at the point of application and the general effects which follow the absorption of the metal into the blood and tissues. The most corrosive salts of any metal are those which are most completely dissociated into ions, that is, the chlorides and nitrates, provided they are soluble. The sulphates are much less irritant because they are less completely dissociated, and perhaps because the sulphuric acid formed hydrolytically may fail to penetrate the cells. The iodides are generally regarded as less irritant than the chlorides, but are less frequently used and less well known.

The least corrosive of the salts of the metals are those formed with the weakly dissociated organic acids, such as the acetates, tartrates or citrates. When these are united with a metal which is not itself an active poison, such as zinc, they are almost purely astringent. The insoluble salts of the metals come into less intimate contact with the tissues and have much less effect. Many of them, however, are slowly taken up and may then act as astringents or irritants. (Cushny, 1924.)

Like the other heavy metals, the soluble salts of zinc precipitate proteins and therefore possess an astringent action, or in large quantities act as irritants and corrosives. The sulphate is the soluble salt most commonly used in medicine, but the chloride, ingested accidentally or with suicidal intent, has frequently given rise to corrosive poisoning. The sulphate is much less irritant and more astringent than the chloride, which is used only as a caustic and disinfectant. The more insoluble zinc oxide (Drinker, Thompson and Marsh, 1927) and the carbonate (Lehmann, 1897) do not cause irritation even when ingested in considerable amounts over long periods of time.

Experimental observations upon the action of organic zinc compounds began as early as 1818, when Devaux and Dejaer (quoted by Helpup, 1889, and by Sacher, 1893) administered zinc acetate and zinc citrate to Spanish prisoners at Lüttich. These early investigators found that large doses of the acetate caused vomiting and mild diarrhea, but that 2 to 4 grams of either the citrate or the acetate were without effect.

Falck (1860, 1861) gave zinc acetate by stomach tube to rabbits (1 to 2 gram doses) and injected zinc acetate in solution into the esophagi of fasting pigeons in doses varying from 0.5 to 2 grams. The rabbits died within 24 hours and displayed at autopsy corrosion and inflammation of the stomach and small intestines. The pigeons exhibited vomiting and diarrhea and, if vomiting was prevented by tying the esophagus above the point of injection, died in from 1½ to 23 hours, depending on the size of the dose. Section showed corrosion of the mucous membrane in the crop and marked inflammation in the upper part of the small intestine. The doses selected by Falck were enormous, especially when one considers the size of his animals and the fact that he was using a soluble salt—so enormous, indeed,

that the whole investigation seems hardly worthy of consideration as a physiological experiment.

Rosow (1862) repeated Falck's experiments upon two pigeons with the exception that his doses of zinc acetate (1 and 1.5 grams) were mixed in the form of a powder with bread and were fed to the pigeons instead of being introduced into the esophagus in solution. The pigeons died in a short time but did not exhibit the vomiting and diarrhea which Falck reported with his birds.

Brandl and Scherpe (1899), investigating the effects of zinc malate, because of their interest in the possible harmful effects of dried apples contaminated with zinc from being dried on zinc trays, fed guinea pigs, rabbits and dogs for periods of 2½ to 4 months small amounts of zinc malate in pill form. With none of the animals on small doses was there any appearance whatever of illness—no loss of appetite, no constipation, no diarrhea, neither during nor after the experiment—and the animals all increased normally in weight. With larger doses, the authors reported local irritation with the occurrence of either diarrhea or persistent constipation.

Salant (1920) reported occasional disturbances of renal function in rats fed daily for 4 months 10 to 15 mgm. of zinc acetate, but no effect upon general health. Cats fed 50 mgm. of zinc daily, as malate, for periods of 10 days to 2 months, also suffered no injury to general health.

The local action of the double salt of sodium zinc tartrate is similar to that of the malate (Sacher, 1893; Brandl and Scherpe, 1899). In small doses it has no untoward effect; in large doses it is irritating to the gastro-intestinal tract, causing, according to these authors, nausea, vomiting and diarrhea, with hyperemia and even ecchymosis and ulceration of the mucosa. Zinc albuminate, however, may be ingested in large daily doses without effect. Rosow (1862), for example, administered zinc albuminate (8 to 9 parts of zinc in 100 parts of the albuminate) without harmful effect to pigeons in daily doses up to 8.5 grams of the albuminate; to dogs in a wide range of dose (2 to 32 grams daily); and to men in doses varying from 3 to 16 grams daily, one patient receiving as much as 278 grams in 36 days without exhibiting digestive symptoms of any sort.

I. Experimental methods. The experiments reported in this paper deal with the results of the feeding of gum acacia suspensions of zinc oxide and solutions of zinc acetate, zinc citrate and zinc malate to rats in daily doses varying from 0.5 to 34.4 mgm. of zinc, and for periods of time varying from 35 to 53 weeks. The investigation was conducted on a total of thirty-five white rats (Mus norvegicus albinus), including thirteen control animals. The rat was selected as an experimental animal because we believe that 9 or 10 months of its relatively short life—one year in a rat's life, as worked out by Donaldson (1924), being approximately equivalent

to 30 years in the human cycle—would give ample opportunity for the appearance of any chronic damage which zinc might be capable of producing. The rats were divided into three groups as follows:

Group I. This group included *a*, eight male rats on varying doses of zinc oxide suspended in 3.5 per cent gum acacia (0.5 to 22.8 mgm. of zinc daily) for from 36 to 43 weeks; *b*, two male rats on zinc acetate solution (1.9 and 3.6 mgm. of zinc daily) for 53 and 48 weeks, respectively; and *c*, two control male rats, one on 3.5 per cent gum acacia solution and one on distilled water, both for 34 weeks. Four of the zinc oxide rats served also as controls of the first 6 weeks of the experiment, receiving during this time distilled water instead of the zinc oxide suspension. Group I rats were caged individually, their fluid intake was measured, the amount of food consumed and its zinc content determined, and all excreta collected and analysed for zinc.

Group II. This group included *a*, three male rats caged together and ingesting for 35 to 36 weeks average daily doses of 34.4 mgm. of zinc in the form of zinc oxide suspended in 3.5 per cent gum acacia solution; and *b*, five male control rats which were caged together and received as their only liquid for 34 to 35 weeks 3.5 per cent gum acacia solution.

Group III. This group included *a*, three males on zinc malate solution (12.4 mgm. zinc per rat per day); *b*, three females on zinc acetate solution (4.4 mgm. zinc daily); *c*, three females on zinc citrate solution (9.7 mgm. zinc daily; *d*, six control rats, three male and three female, on distilled water —all for 29 weeks. At the end of this period, group III rats were mated and continued on zinc, but some not on their original solutions, for from 6 to 18 weeks longer. The controls were also mated and continued on distilled water. Before mating, group III rats were caged together in groups of three, each group being of the same sex.

The rats in groups I and II were a very pure healthy stock obtained from a Philadelphia colony bred expressly for experimental purposes. Group III rats were of a different and less pure strain, some of the original rats and many of their offspring showing pale brown markings. Group III rats tended also to reach a higher maximum weight than did the rats in groups I and II.

Group I rats were put on zinc at the age of 12 weeks; group II rats at 22 weeks, and group III rats at 5 to 7 weeks. Previous to zincing, all groups had a preliminary adaptation period on the diet used during the experimental period. During the course of the experiment, each rat was weighed weekly or fortnightly; at the conclusion of the experiment each rat was autopsied, gross and microscopic studies and chemical analyses for zinc being made on all tissues. The exact zinc dosage, the form of the dose, and the period during which zinc was administered to each rat in the three groups may be seen in table 1.

TABLE 1

Summary of amounts and duration of zinc doses and of blood findings just before autopsy in three groups of zinced rats

RAT GROUP AND NUMBER	FORM OF DAILY DOSE	AVERAGE DAILY DOSE CALCULATED AS ZINC	NUMBER OF WEEKS ON ZINC	BLOOD FINDINGS		
				Per cent hemoglobin	Red blood cells	White blood cells
		mgm.				
Group I						
2	Zn acetate	3.6	48	90	10,168,000	13,400
3	Zn acetate	1.9	53	90	9,857,000	12,300
1	Zn oxide	0.5	42	90	9,673,000	21,200
4	Zn oxide	2.7	42	100+	8,032,000	11,800
5	Zn oxide	5.4	42	90	9,862,000	7,600
6	Zn oxide	7.0	43	90	9,537,000	7,200
7	Zn oxide	10.4	39	90	10,071,000	9,100
8	Zn oxide	13.7	37	90	12,013,000	11,400
9	Zn oxide	17.9	36	90	11,797,000	11,300
10	Zn oxide	22.8	38	90	11,464,000	8,500
11 (Control)	3.5 per cent gum acacia	0	34	95	11,536,000	16,000
12 (Control)	Distilled water	0	34	85	10,116,000	17,100
Group II						
13	Zn oxide	34.4	35	85	12,269,000	10,800
14*	Zn oxide	34.4	36			
15	Zn oxide	34.4	35	85	10,921,000	7,000
16 (Control)	3.5 per cent gum acacia	0	34	80	10,626,000	6,300
17 (Control)	3.5 per cent gum acacia	0	34	80	10,824,000	15,500
18 (Control)	3.5 per cent gum acacia	0	34	95	12,852,000	11,800
19 (Control)	3.5 per cent gum acacia	0	34	90	11,967,000	9,300
20 (Control)	3.5 per cent gum acacia	0	35	90	11,356,000	9,900
Group III†						
21	Zn malate	12.4 16.5	29 12	90	11,335,000	13,500
22	Zn malate	12.4 11.1	29 13	100	9,808,000	8,800
23	Zn malate Zn acetate	12.4 6.3	29 18	90	9,033,000	12,500

TABLE 1—*Concluded*

RAT GROUP AND NUMBER	FORM OF DAILY DOSE	AVERAGE DAILY DOSE CALCULATED AS ZINC	NUMBER OF WEEKS ON ZINC	BLOOD FINDINGS		
				Per cent hemoglobin	Red blood cells	White blood cells
		mgm.				
24	Zn citrate	9 7	29			
	Zn malate	11 1	14	90	9,727,000	17,900
25	Zn citrate	9 7	29			
	Zn oxide	13 0	14	100+	11,529,000	7,500
26	Zn citrate	9 7	29			
	Zn acetate	2 0	14	90	10,128,000	17,300
27	Zn acetate	4 4	29			
		6 3	18	90	9,816,000	10,600
28	Zn acetate	4 4	29			
	Zn malate	16 5	13	80	9,125,000	11,300
29*	Zn acetate	4 4	29			
	Zn oxide	13 0	4			
	Zn acetate	3 5	2			
30 (Control)	Distilled water	0	41	90	9,637,000	16,000
31‡ (Control)	Distilled water	0	43	100	8,938,000	9,800

* Died of intercurrent respiratory disease.

† The experimental life of group III rats was divided into two parts, the one previous to, the other following mating. The zinc doses and, in many cases, the zinc compound administered was changed when the second period began—a fact which explains the double dosing in this group.

‡ Four other water controls were included in group III but are omitted from this table since no blood counts were made on them.

HOUSING AND METHOD OF COLLECTING EXCRETA: Group I rats were housed in individual cages of the type shown in figure 1. This type of cage, together with our method of collecting excreta, is a slightly modified copy of an apparatus and a method demonstrated to us by Dr. James L. Gamble of the Children's Hospital, Boston—an apparatus resulting from a number of alterations by Doctor Gamble and by Dr. P. G. Shipley of Baltimore, of a metabolism cage for rats originally described by Ackroyd and Hopkins (1916). In order to prevent zinc contamination, the cages were of pure copper, all unions being made with copper rivets instead of solder. Every few months, after careful cleaning with acid, and drying,

the cages were heavily lacquered with a nitrocellulose lacquer and then baked. This was done in order to prevent the formation of the green basic carbonate with which the parts of the cage exposed to wetting tended to become spotted.

A in figure 1 is a tunnel, partially obstructed inside by a small solid fence (on the inside of the tunnel at point *B*), over which only the rat's head can go, and leading to a detachable food can, *C*. This arrangement of the food compartments prevents gross contamination of the excreta with food and, because of the detachable can, provides an easy method of weighing the food supplied and food residues. A calibrated burette, *D*, contains the solution or suspension of zinc to be administered and provides the only source of liquid. The rat is thus forced to consume a daily dose of zinc, the amount of which varies with the concentration of the suspension. After 2 or 3 weeks' observation, one can estimate closely how much of a given suspension a rat will drink per day, the average daily consumption being remarkably uniform. Though one cannot by this method administer a selected exact dose, one can, by varying the strength of the suspension offered, ensure that the rat will consume an average daily dose very close to the selected one. *E* is a detachable wire mesh floor which rests upon supports hanging from the bottom of the cage.

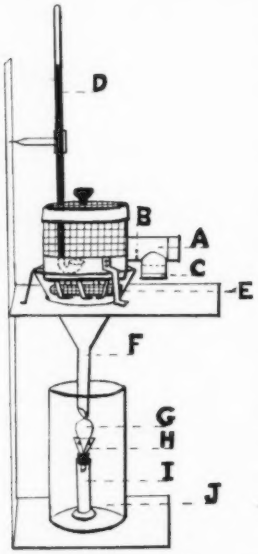


Fig. 1. Diagram of apparatus used in quantitative collection and separation of rat urine and feces. See text for explanation of letters.

The cage rests on a large funnel, *F*, down which the urine runs onto a pear-shaped glass bulb, *G*, which is supported about $\frac{1}{4}$ inch clear of all its surfaces in a small glass funnel, *H*, by means of a few glass spicules projecting from its sides. Urine reaching this bulb flows down the funnel into a small graduate, *I*, containing a little powdered thymol. The daily urine volume is thus self-registered. The amount of food consumed is determined by weighing the food can and its contents at the beginning and at the end of a given period, and, similarly, the fluid consumption by burette readings.

The mesh of the wire floor is sufficiently large to allow rat feces to fall through without sticking. The falling fecal masses strike the sides of the large funnel, pass down the funnel stem, and strike the surface of the glass

bulb beneath. Being formed and dry, they bounce off into the large surrounding glass cylinder, *J*, and are collected weekly.

All of the apparatus used in our experiments was carefully washed with hot distilled water weekly and the washings—chiefly food dust, hair and some dried urine—saved and analysed for zinc. All apparatus was then thoroughly cleaned with soap and water, and either a new collection period started or the collection period under way at the time continued, as the case might be.

At first analyses were made of the zinc content of a week's excreta from group I rats. After 10 weeks, however, the collection periods were lengthened to 2 and, finally, to 4 weeks.

DIET. *Group I rats.* The composition of the stock food was as follows:

	Parts
Rolled oats	5.0
Hominy	3.0
Dried milk powder (Klim)	1.5
Beef scraps	2.5
Salt	trace
Yeast	0.36

Each rat also received 5 grams of lettuce twice weekly. Whenever a new stock food was made up, two 100-gram samples were analysed for zinc. The average figure obtained was 0.059 mgm. of zinc per gram of food. Sample analyses of lettuce gave an average zinc concentration of 0.0013 mgm. of zinc per gram. With these data and with the record which was kept of the food consumed by each rat in group I, the amount of zinc ingested by each individual with his food could be determined, and added to his zinc dose to obtain a figure for the total zinc ingested.

Groups II and III rats. These rats received the same stock food as group I rats, except that yeast was omitted. They also received molasses horse feed (a mixture of alfalfa, corn, oats and molasses) and dog biscuit daily, with lettuce twice weekly.

METHOD OF PREPARATION AND ADMINISTRATION OF ZINC DOSES. Zinc was administered in liquid form, rather than as a dry powder mixed with the food, in order to reduce the danger of contaminating with zinc the excreta of group I rats.

Zinc oxide suspensions. A primary stock suspension was made by suspending about 200 grams of pure zinc oxide[1] in approximately 5 liters of 10

[1] Chemical analysis of the zinc oxide used in these experiments gave the following results:

	per cent
Insoluble in HCl	0.007
CO_2	0.025
Total S as SO_3	0.001

per cent zinc-free gum acacia solution. This was decanted several times, and the final supernatant liquid, now approximately 3.5 per cent gum acacia, was analysed in order to obtain its exact zinc concentration per cubic centimeter.

If a fortnightly collection period was running for group I rats, the amount of fluid a rat would drink in this length of time was liberally estimated—about 250 cc. Into this volume of 3.5 per cent gum acacia solution was introduced the number of cubic centimeters of the primary stock suspension that would supply an amount of zinc amply sufficient to give the rat concerned a daily dose of zinc within the range allotted to him.

From these secondary stock suspensions, the graduated siphon burettes in each cage were filled whenever necessary, meticulous care being exercised to avoid any loss of zinc in the process. Readings were then made of each rat's daily fluid consumption. In order to obtain accurate figures for the amount of zinc actually ingested by each group I rat, all of the suspension left at the end of a collection period in the burette and in the secondary stock bottle was analysed, and the figure obtained was subtracted from the accurately known zinc allowance made up for the rat in question. Group II rats were supplied in a similar manner.

Organic zinc salt solutions. The salts concerned (acetate, citrate and malate) were synthesized in this laboratory by Dr. Lawrence Fairhall, were then made up in stock solutions of known zinc content, and were administered in graduated siphon burettes, the amount of zinc ingested being calculated from the fluid consumption.

In groups II and III one burette supplied three rats, so the dose figures for individuals in these groups represent one-third of the average total amounts of zinc ingested by all members of the group.

URINALYSES AND BLOOD COUNTS. Collections of urine for urinalysis were made from group I rats eight or nine times during the course of the investigation, each time immediately after the apparatus had been thoroughly cleaned for the week. During the period of the collection—at first 5 to 6 hours, later 18 hours—the rats were given no food so that there

Cl	0.004
PbO	0.023
CdO	0.004
Bi_2O_3	under 0.001
CuO	under 0.001
Fe_2O_3	under 0.001
MnO	under 0.001
As_2O_3	under 0.001
Sb_2O_3	under 0.001
ZnO (Difference)	99.799
Reducing power as SO_2	0.010
Water soluble salts dried at 110°C	0.098

could be no chance of dust contamination from this source. The burettes, however, were filled as usual. In this manner, with the longer collection period, approximately 5 cc. of uncontaminated urine were obtained from each group I rat. This was tested qualitatively for albumin with the sulphosalicylic acid test and for sugar with Benedict's reagent. When albumin was determined quantitatively, Esbach's reagent was used.

Just before autopsy, blood for red and white cell counts and for hemoglobin determinations was obtained from the rat's tail vein. Counts were made with pipettes standardized by the U. S. Bureau of Standards.

Autopsies. Immediately preceding autopsy, the animal was anesthetized with chloroform and then at once decapitated over a weighed porcelain evaporating dish. The blood, which poured freely from cut vessels in the neck, was weighed and used for zinc analysis.

The rat was then skinned, particular care being taken to make the removal of the hide complete since, in zinced rats, incomplete removal around the feet was a possible source of zinc contamination of the carcass. Hides, after weighing, were thoroughly washed in distilled water to remove any extraneous zinc that might be adherent. The viscera were next removed from chest and abdomen, the gastro-intestinal tract from the throat down being taken out intact, slit up, and the contents washed out with running water. In the case of zinced animals, the mouth and throat were also washed out with water. All organs were dissected clean and weighed in tared evaporating dishes, small sections of each being taken for histological study. Of the reproductive tract, only the glandular tissues were analysed, i.e., the testes, seminal vesicles and accessory glands in the male, and the ovaries in the female. A 10-gram sample of muscle was removed from the hind quarters, and, finally, the brain and cord were removed. The rest of the rat—practically all skeleton and muscle, with an insignificant amount of tissues not considered worth analysing, such as bladder, omentum, blood vessels, etc.—was weighed and analysed *en masse* under the name of "carcass."

Chemical analysis. All samples were dried, then charred on a hot plate and ashed in a muffle furnace at about 450°C.

Analysis of zinc oxide doses and of dose remains. The ash of samples of zinc oxide doses and the ash of the dose remains were dissolved in 5 cc. of 1:1 hydrochloric acid and 20 cc. of water and titrated with potassium ferrocyanide solution.

Analysis of feces and rat carcasses. The ash of feces and of carcasses was treated with 1:1 hydrochloric acid and the residue returned to the muffle repeatedly until all the carbon had disappeared. Iron was removed in acid solution by cupferron. On account of the excessive amount of calcium present, the filtrate was made up to large volume—about 600 cc. It was then neutralized with ammonium acetate, and the zinc and added

copper brought down as sulphides. (If any calcium phosphate was in evidence at this stage, it was necessary to reprecipitate.) These sulphides were well washed with water, then several times with hot alcohol, and were finally dissolved in nitric acid. The latter was thoroughly fumed off, the residue taken up with hydrochloric acid and water, and the copper removed as sulphide. The filtrate containing the zinc in pure form was evaporated to dryness and titrated as described above.

Analysis of food and hides. The method of analysis of food samples and of hides was the same as for feces and carcasses, except that the neutralized filtrate after the removal of iron with cupferron was made up approximately to 100 instead of to 600 cc.

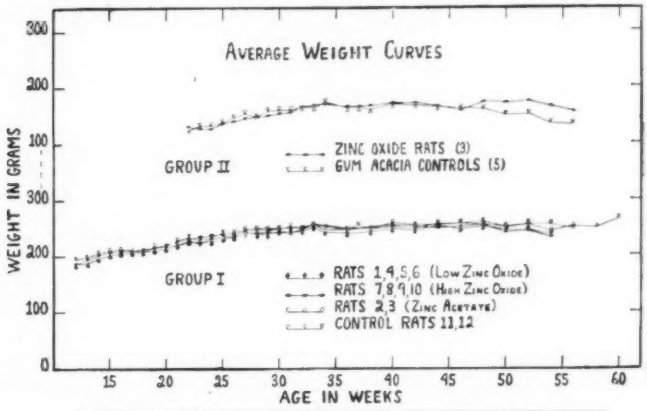
Analysis of urine and tissues. The analytical procedure in the case of urine and tissues was the same as with food and hides until the last stage was reached. At this point the pure zinc residue was dissolved in 10 cc. of water. Zinc determinations on the tissues were made by the ferrocyanide turbidimetric method; on the urines, for the most part, by the urobilin colorimetric method (Lutz, 1925). A detailed description of the above procedures has been given by Fairhall (1926).

II. Results of clinical and laboratory observations and of autopsy findings. *General health and weight.* All of the rats continued in good health until June, the experiments having begun for the most part in December. During the summer, however, a chronic respiratory infection—the first symptom of which was a noisy respiration, a sort of croaking—gradually spread through the colony, affecting approximately half of both the control and the zinced rats. Gradually some loss in weight occurred, relatively little in some rats, greater in others. Two rats eventually died of this disease, the diagnosis at autopsy being chronic bronchopneumonia. The gum acacia control rats in group II were earlier and rather more severely affected than any of the zinced rats, the next most severely involved lot being the zinc oxide rats in group II, which were housed in an adjoining cage. Group I rats were later and to a less extent involved.

Except for this final pneumonia epidemic, the health of all of the rats was normal throughout the 34 to 53 weeks of the experiment. The animals showed normal appetite and activity, healthy lustrous fur and normal gain or normal maintenance of weight. There were no signs of intestinal or of nervous disturbances. The weekly food and liquid consumption of individual rats remained astonishingly uniform throughout the experimental period. The zinced rats were as healthy and gained weight as rapidly or maintained it as normally as did the controls. As a result of the unpalatability of the zinc doses, however, especially the higher ones, the zinced animals were inclined to drink less than the controls.

In figures 2 and 3 the weight curves of various groups of zinced rats are

compared with similar curves from control animals. It is obvious from these figures that in so far as weight gain and weight maintenance are concerned the zinced rats were the peers of the control animals. The



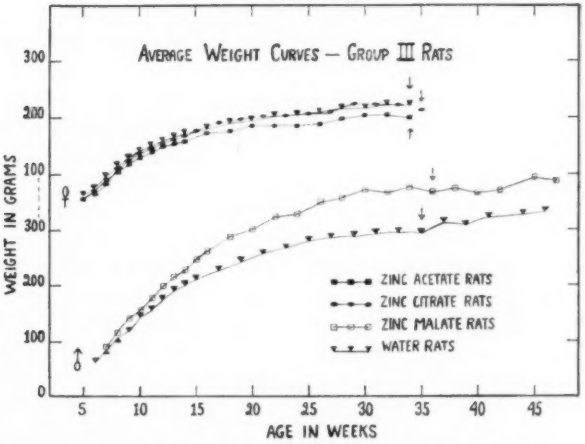


Fig. 2 (Upper). Average weight curves of rats in groups I and II.

Fig. 3 (Lower). Average weight curves of rats in group III, each curve representing the average of three rats. Arrows indicate points at which animals were mated and fertility experiments begun.

final respiratory epidemic explains the gradual falls shown in figure 2 in the average weight curves of the gum acacia controls during the last 10 weeks of the experiment and of the zinc oxide rats (group II) during the

last 4 weeks. Some of the weight curves of the rats in group I also show at the very end, but to a less marked degree, the effect of the epidemic.

BLOOD FINDINGS. Table 1 shows the blood findings on each rat just before autopsy. The following tabular statement presents the extreme and the average red cell and white cell counts and hemoglobin determinations in the zinced rats as compared, not only with figures from similar observations upon our control animals, but also with blood studies reported by Goodall (1909) upon a series of normal rats.

	Extremes	*Averages*
	Red Cells	
Zinced rats	8,032,000–12,269,000	10,308,000
Control rats	8,938,000–12,852,000	10,872,000
Goodall's normals for rats		8,100,000
	White Cells	
Zinced rats	7,000–21,200	11,600
Control rats	6,300–17,100	12,400
Goodall's normals for rats		10,600
	Per Cent Hemoglobin	
Zinced rats	80–100+	90 5
Control rats	80–100	89 4
Goodall's normals for rats		110 0

It is clear from these figures that the ingestion of zinc, even in large doses over long periods of time, causes no significant variation from the normal in the blood picture of rats.

URINALYSES. During the course of the experiment, eight or nine analyses were made of the urine of each of the rats in group I. Albumin is a normal finding in rat urine—an Esbach quantitative analysis, for example, showed 0.3 gram per liter in a 24-hour specimen of the urine of control rat 11—and was always present in the urine of both control and zinced rats, the amount varying from a faint trace to a distinct cloud. In the case of two rats, however,—zinced rat 7 and control rat 12—there was always a heavy precipitate of albumin with the sulphosalicylic acid test. Neither of these rats exhibited any untoward clinical symptoms ascribable to the kidney, and both showed normal kidneys at autopsy. Sugar was never found and casts were so few and infrequent as to be of no significance.

The weekly urine volume varied with different rats depending upon the fluid intake, the heavily zinced rats drinking much less of the unpalatable suspensions which provided their only source of fluid, than did the control and low dose zinc rats, and excreting, consequently, less volume of urine. During the entire course of the experiment, however, the volume, both of fluid intake and of urine output in individual rats, remained remarkably constant; significant variation did not occur in a single instance.

AUTOPSY FINDINGS. *Gross findings.* With the following exceptions, which are plainly due to intercurrent disease, the gross autopsy findings were negative. The lungs of zinced rats 9, 13, 15, 21, 25, 26 and 27 and

of control rats 11, 18 and 20 showed small consolidated areas and pus sacs, mainly confined to the upper lobes. In some instances adhesions to the pleura were present. In zinced rats 7, 8, 14 and 29 and in control rats 17 and 19 these processes were more extensive, being present in patches throughout the lungs. Rat 3 showed a pus pocket at the external meatus of the bladder which exuded a light green pus when the skin was cut. Parasitic cysts were found in the livers of rats 3, 8 and 14.

Histological findings. The histological findings in the lungs of the rats reported above as showing gross changes varied in degree, extent and stage of disease process, but in all instances was there evidence of bronchopneumonia or its sequelae. Many sections showed only small patches, others larger areas of solid leucocytic infiltration; other sections showed atelectatic cavities and dilated bronchi filled with exudate; still others showed definite encapsulated, chronic abscesses, which undoubtedly had their origin in pneumonic foci. The livers of rats 14 and 21 showed some cloudy swelling, a degree of disturbance no more than one might expect from the infection in the lung.

With the exceptions noted, histological studies upon the organs of the zinced rats revealed, throughout, normal tissues. Sections of stomach, intestines, kidney, pancreas, spleen, adrenal and reproductive glands were normal in all instances and, with the exception of the two cases of cloudy swelling already mentioned, the livers of all zinced rats were normal.

In brief, we may say of our autopsy findings, that approximately one-half both of control and of zinced rats showed evidence of chronic bronchopneumonia or its sequelae, the degree, extent and stage of disease process varying in different animals. With the exception of these evidences of bronchopneumonia or its consequences and of parasitic cysts in three livers, gross and microscopic autopsy studies showed normal organs in all of the zinced rats. In no instance was there any evidence of damage due to zinc.

Summarizing our clinical and laboratory observations upon zinced rats, we may say that we have never observed any significant clinical symptom nor obtained any significant laboratory evidence, either during life or from autopsy findings, of damage resulting from the long-continued daily ingestion of zinc oxide or of zinc acetate, citrate or malate. These results are in accord with our experiences, already reported, upon the long-continued feeding of zinc oxide to cats and dogs (Drinker, Thompson and Marsh, 1927).

III. Zinc excretion. *Zinc in normal rat urine and feces.* The normal amount of zinc in the urine (not including cage washings) of rats on an ordinary mixed rat diet, such as the one used by us, ranges approximately between 0.02 and 0.20 mgm. per week; that in rat feces, between 1.25 and 7 mgm. It must be remembered, however, in connection with the figure

TABLE 2

Specimen figures of zinc ingested weekly in food and of weekly zinc excretion in urine and feces of normal rats on a constant quality diet

RAT NUMBER	EXPERIMENTAL WEEK*	TOTAL ZINC INGESTED IN FOOD (CALCULATED)	ZINC EXCRETED			
			In urine	In feces	In cage washings†	Total†
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
7	4	3.6	0.12	3.3		3.42
	5	3.2	0.10	3.0		3.10
	6	3.6	0.15	3.9		4.05
		10.4				**10.57**
8	5	3.4	0.10	3.0		3.10
	6	3.6	0.15	3.9		4.05
		7.0				**7.15**
9	4	3.5	0.04	3.3		3.34
	5	3.2	0.07	3.0		3.07
	6	3.6	0.12	3.3		3.42
		10.3				**9.83**
10	4	3.3	0.02	3.3		3.32
	5	3.1	0.02	3.3		3.32
	6	3.6	0.12	3.3		3.42
		10.0				**10.06**
11	13 and 14	10.6	0.14	8.8	0.08	9.02
	15 and 16	10.4	0.09	9.9	0.13	10.12
	17 and 18	10.6	0.19	13.6	0.09	13.88
	19 and 20	10.4	0.07	10.0	0.20	10.27
	21 and 22	11.5	0.07	10.9	0.20	11.17
	23 and 24	11.7	0.10	10.9	0.20	11.20
	25 and 26	9.6	0.10	9.1	0.20	9.40
		74.8				**75.06**
12	11 and 12 (9 days only)	6.7	0.05	6.4	0.03	6.48
	15 and 16	10.6	0.11	9.9	0.09	10.10
		17.3				**16.58**

* Experimental collection periods lasted at first a week, then 2 weeks, then 3, and finally 4 weeks. Note that figures upon rats 11 and 12 cover 2-week periods.

† For the first 6 weeks of the investigation, zinced rats, 7, 8, 9 and 10 were used as controls, receiving distilled water instead of zinc oxide suspensions. During this time, however, cage washings were not saved for zinc analysis, so a small fraction of the zinc excreted by these rats during this time is not included in the table. From the seventh week on, all cage washings were saved.

TABLE 3

Specimen figures of zinc ingested and zinc excreted by rats on varying daily doses of zinc oxide

RAT NUMBER	EXPERIMENTAL WEEK	ZINC INGESTED		ZINC EXCRETED			TOTAL ZINC INGESTED	TOTAL ZINC EXCRETED
		In dose	In food	In urine	In feces	In cage washings		
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	11 and 12	5.6	9.0	0.15	15.8	0.29	14.6	16.24
	13 and 14	4.6	9.6	0.09	16.8	0.41	14.2	17.30
	15 and 16	5.7	9.2	0.14	15.4	0.46	14.9	16.00
	17 and 18	8.5	9.6	0.18	14.1	0.26	18.1	14.54
	19 and 20	8.5	10.3	0.12	17.3	0.32	18.8	17.74
	21 and 22	5.7	9.7	0.12	15.1	0.32	15.4	15.54
	23 and 24	6.6	11.0	0.13	15.9	0.37	17.6	16.40
	25 and 26	6.9	9.6	0.13	15.8	0.37	16.5	16.30
	27, 28 and 29	8.2	13.8	0.20	20.9	0.54	22.0	21.64
							152.1	**151.70**
4	11 and 12	38.0	9.6	0.13	43.7	0.29	47.6	44.12
	13 and 14	35.3	10.5	0.14	49.8	0.54	45.8	50.48
	15 and 16	31.5	9.8	0.17	43.1	0.40	41.3	43.67
	17 and 18	41.3	10.2	0.21	43.0	0.39*	51.5	43.60
	19 and 20	35.9	9.5	0.14	47.9	0.39	45.4	48.43
	21 and 22	44.2	10.3	0.14	53.6	0.39	54.5	54.13
	23 and 24	36.4	10.8	0.18	47.3	0.39	47.2	47.87
	25 and 26	34.1	9.9	0.18	42.7	0.39	44.0	43.27
	27, 28 and 29	50.2	13.6	0.20	60.9	0.57	63.8	61.67
							441.1	**437.24**
5	13 and 14	73.8	10.3	0.26	88.2	0.38	84.1	88.84
	15 and 16	71.1	10.3	0.17	90.0	0.86	81.4	91.03
	19 and 20	74.9	10.3	0.14	91.3	0.29	85.2	91.73
	21 and 22	76.0	10.7	0.14	85.4	0.29	86.7	85.83
	23 and 24	65.4	10.9	0.16	69.9	0.39	76.3	70.45
	25 and 26	67.1	9.9	0.16	70.5	0.39	77.0	71.05
	27, 28 and 29	98.5	14.0	0.20	116.4	0.52	112.5	117.12
							603.2	**616.05**
10	11 and 12	342.0	9.3	0.31	304.1	0.79	351.3	305.20
	13 and 14	324.0	9.7	0.20	344.0	0.49	333.7	344.69
	15 and 16	312.0	9.9	0.24	357.5	0.40	321.9	358.14
	19 and 20	333.7	9.9	0.30	331.8	0.77	343.6	332.87
	21 and 22	332.7	10.4	0.29	350.0	0.77	343.1	351.06
	23 and 24	303.7	11.0	0.30	374.5	0.60	314.7	375.40
	27, 28 and 29	444.0	13.6	0.20	388.0	0.63	457.6	388.83
							2465.9	**2456.19**

* Analysis lost. Figure estimated.

for zinc in urine that always some urine dried on the wire of the cage floors and on the funnels, with the result that its zinc content was included in the figure for cage washings instead of in urinary zinc. Actually, therefore, the normal total weekly excretion of zinc in urine by rats ranges somewhat higher than 0.02 to 0.20 mgm. We have not added the figure for cage washings to that of urinary zinc to secure a figure for total zinc in urine because of the fact that, undoubtedly, at times the cage washings included not only dried urine but also small fragments of feces which had stuck to the wire mesh of the floor and which could be completely removed only in the final washing. Table 2 gives specimen figures of the amounts of zinc ingested weekly in food by six normal rats during control periods, and of the amounts of zinc excreted in urine and feces by these same rats during the same periods. It is obvious from these figures that the great bulk of zinc normally passing through the body is excreted through the gastro-intestinal tract, a very minor amount through the kidneys. This observation on rats merely confirms previous ones on cats, dogs and men (Drinker, Thompson and Marsh, 1927; Drinker, Fehnel and Marsh, 1927).

Table 2 also shows that while the weekly excretion of zinc may lag a little behind or may somewhat exceed the weekly ingestion of zinc, nevertheless, during longer periods of time—several weeks—the total amounts of zinc ingested and of zinc excreted by adult rats are practically the same. Group I rats upon which these observations were made were 12 weeks old and between 180 and 200 grams in weight at the beginning of the experiments. We have no similar data upon very young, very rapidly growing rats. It is highly probable, however, that in the early weeks of life there is a considerable excess of zinc intake over zinc excretion, since the zinc concentration of rats per gram of animal tends to be the same, regardless of age (Drinker and Collier, 1926).

Zinc in the urine and feces of zinced rats. Table 3 gives specimen figures of the amounts of zinc ingested in food and in zinc doses of varying amounts by four zinced rats during total experimental periods of 15 and 19 weeks. The same table also contains data upon the amounts of zinc excreted in the urine and feces and in the cage washings of these same rats during the same experimental periods.

A number of significant facts are to be derived from this table. Considering the figures upon zinc ingested in food, for example, as an index of the total amount of food eaten, we have in them evidence that the daily ingestion of large doses of zinc had no effect upon the total food consumption. Rat 10, for example, on an average weekly dose of 159.6 mgm. of zinc, consistently ate as heartily as rat 1 on an average weekly dose (3.5 mgm.) less than the normal zinc content of his food. Had digestive disturbances existed in the highly zinced rats, this clearly would not have been the case. The uniformity of the weekly amounts of zinc ingested in

food by individual rats, indicating as it does uniform weekly total food consumption, is also graphic evidence of good health. The quantity of food eaten depended on the rat's appetite; he was always provided with an excess. Had the zinc been a source of gastro-intestinal irritation, undoubtedly as the experiment progressed there would have been a decrease in the amount of food consumed by individuals, especially by the highly zinced animals.

The figures in table 3 show clearly that the gastro-intestinal tract in rats is the chief avenue of excretion of abnormal amounts of ingested zinc, as it is the main route of excretion in the normal zinc metabolism. Highly zinced rats show increases over the normal in urinary zinc but these increases are relatively small, much smaller than is the case with highly zinced cats and dogs. Rat 10, for example, during 3 control weeks on distilled water, averaged 0.053 mgm. of urinary zinc and 3.3 mgm. of fecal zinc per week. During 15 experimental weeks on an average weekly zinc dose (for these weeks) of 159.5 mgm., the zinc in this rat's urine averaged 0.12 mgm. per week; in his feces, 163.3 mgm.—that is to say, urinary zinc increased 2.26 times, fecal zinc 49.5 times. Cats and dogs on doses of zinc even less per kilogram of animal than the dose of rat 10, showed average increases in urinary zinc of approximately three, five and even seven times the amount normally excreted in 24 hours. Similarly, zinc in the urine of zinc workmen, exposed to the inhalation of zinc oxide and of fine metallic zinc dust, shows a greater percentage increase over the normal level than occurs in the urine of zinc-fed rats (Batchelor, Fehnel, Thompson and Drinker, 1926).

Whether these differences in the excretion of zinc by the kidneys in cats, dogs and men, as compared with rats, are due to different functional capacities of the kidneys in the respective species—the normal presence of albumin in rat urine should be considered in this connection—or whether a higher concentration of hydrochloric acid in the stomachs of the carnivora ensures the solution and absorption of more of the ingested zinc than occurs in the herbivora, with a consequently greater proportional increase in the urinary zinc, we are not prepared to say. If the latter factor be the determining one, a large fraction of the zinc oxide ingested by rats must pass unabsorbed, possibly unchanged, through the gastro-intestinal tract.

The data in table 3 upon total zinc ingested and total zinc excreted by four representative rats on varying zinc doses, during illustrative periods of 15 and 19 weeks, show that rats are able to excrete as much zinc as they ingest, even when the zinc dose is very large. We are not able to present ingestion and excretion data upon individual animals for 10 consecutive months, although the experiments were carried on for that length of time. Accidental contamination or loss of occasional specimens during the course of collection or analysis was inevitable during so long a period, and pre-

vented our obtaining an unbroken series of figures. It is clear, however, from the large number of excretion figures which we have—specimens of which are presented in table 3—that rats do not tend to store zinc in amounts significantly above the normal, but that their excretion of this metal tends to keep pace with its ingestion. The next section contains further data upon the question of zinc storage.

In summary, we may say that our evidence upon the excretion of zinc by rats indicates an insignificant normal excretion of zinc by the kidney, with a relatively small increase in urinary zinc following the ingestion of even very large amounts of the metal. The great bulk of ingested zinc, both in the normal metabolism and following zinc dosing, is excreted by the gastro-intestinal tract. Excretion of zinc in rats tends to keep fairly close pace with ingestion—a fact true whether the ingested zinc be an organic compound or the more insoluble zinc oxide.

IV. Zinc storage. In figure 4 the average zinc concentrations of the different tissues and of the entire bodies of seven control rats are compared with the zinc concentrations of eleven rats fed varying doses of zinc oxide daily for from 35 to 43 weeks and with the zinc concentrations of eleven rats fed daily doses of various organic zinc compounds for from 35 to 53 weeks. Zinc concentrations are expressed in milligrams per gram of tissue.

It should be mentioned here that, in general, our figures for normal zinc concentrations in rat tissues, obtained by the turbidimetric method of analysis, average somewhat higher than those reported by Lutz (1926) with his urobilin method. We have repeatedly found instances of concentrations within the ranges reported by Lutz but, in the main, the range of our figures is more extensive, especially at the upper end, and our average normal figures higher. For this reason, we include the following tabular statement of the average zinc concentrations obtained by us in the tissues of seven control rats:

	Mgm. zinc per gram of tissue
Blood	0.007
Hide	0.036
Muscle	0.038
Carcass (bone and muscle)	0.041
Gastro-intestinal tract	0.045
Liver	0.047
Lungs	0.048
Kidneys	0.050
Brain and cord	0.053
Testes and seminal vesicles	0.082
Ovaries	0.090
Pancreas	0.173
Adrenals	0.180
Spleen	0.228
Total rat	0.040

The significant facts upon zinc storage in rats brought out in figure 4 may be summed up as follows:

The ingestion of zinc oxide or of organic zinc compounds by rats, over long periods of time and even in large amounts, does not significantly increase the zinc concentration in the tissues and organs of this species of animal. The only increases worth commenting on are the increase in the zinc concentration of the blood of rats ingesting organic zinc compounds and the increase in the zinc concentration of the kidneys of the zinc-fed animals. The other increases shown in figure 4 are trifling and would probably disappear if the series were larger.

The blood of the organic zinc salt rats, with a single exception, was always higher in zinc than was the blood of control animals or of rats fed

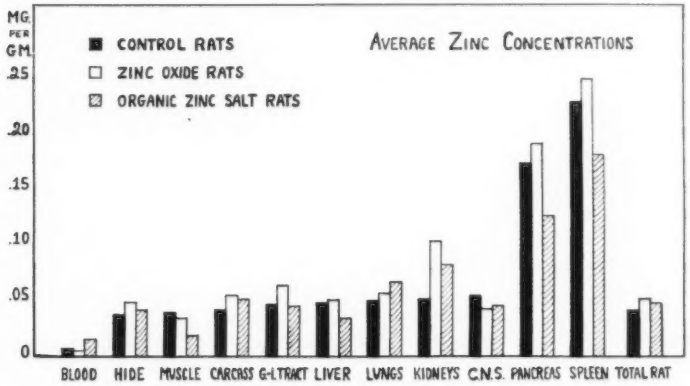


Fig. 4. Final average zinc concentrations in tissues and organs of control rats and of two groups of rats fed, the one, zinc oxide, the other, organic zinc salts, over a long period of time.

zinc oxide, the average blood zinc concentration of the organic salt rats being 0.015 mgm. of zinc per gram of blood as compared with 0.007 for the control rats. The zinc oxide rats were uniform at the lower end of the normal range, their average blood zinc concentration being 0.005 mgm.

This increase in the blood zinc of the organic salt rats suggests the probability that proportionally more of the organic zinc compounds were actually absorbed than was the case with the oxide. Assuming this fact to be true, it did not, however, promote zinc storage since the organic zinc salt rats showed, in general, a somewhat lower zinc concentration of tissues than did the zinc oxide rats.

The increase in the zinc concentration of the kidneys of the zinc-fed animals is no more than one would expect to find in an organ concerned in

the excretion of zinc. Our experience with cats and dogs (Drinker, Thompson and Marsh, 1927) leads us to believe that this increase would disappear in a short time were zinc feeding discontinued. One might expect to find a decided increase in the zinc concentration of the gastro-intestinal tract since this tract, rather than the kidney, is the chief organ of excretion of zinc. Whether the relatively slight increase which does occur—and this only in the zinc oxide-fed animals—is to be explained by a rapid passage of zinc through the gastro-intestinal walls and a consequently relatively small increase in tissue zinc concentration at any one time, or whether it is due to the washing of the tract necessary before analysis in order to get rid of intestinal contents and possible contamination from unabsorbed zinc, we are unable to say.

The concentration of the total rat in the case of the zinc-fed animals is slightly but not significantly greater than that of the controls (controls, 0.040 mgm. of zinc per gram of rat; zinc oxide rats, 0.050 mgm.; organic zinc salt rats, 0.046 mgm.). In terms of total zinc content, this increase represents a few milligrams of zinc only—a total increase of 2.5 mgm., for example, in a 250-gram rat, which is less than the daily dose of all except two of the zinced animals. Trifling increases of this sort in a normal constituent of all body tissues are clearly not significant in terms of storage, especially when one takes into account the many months of zinc feeding. The smallness of the increase in total zinc concentration in the zinc-fed animals is the more noteworthy in that the daily doses of zinc were, in many cases (see table 1), very large in proportion to the size of the animals.

SUMMARY AND CONCLUSIONS

Daily doses, ranging from 0.5 to 34.4 mgm. of zinc, in the form of gum acacia suspensions of zinc oxide and of aqueous solutions of zinc acetate, zinc citrate and zinc malate have been administered by mouth to rats for periods of time varying from 35 to 53 weeks. Clinical and laboratory studies made upon these animals include observations upon general health during the course of the experiment (weight determinations, appetite, nervous and digestive symptoms, condition of fur, etc.); amount of food and of fluid intake; volume of urine output; urinalysis; hemoglobin and blood cell determinations; gross and microscopic examination of tissues following autopsy; and, finally, determinations of ingested zinc, of zinc excreted in urine and feces, and of zinc content of organs and tissues after death.

As a result of these studies, we have never observed any significant clinical symptom nor obtained any significant laboratory evidence of damage resulting from the daily ingestion by rats, during long periods of time, of zinc oxide, zinc acetate, zinc citrate or zinc malate. Gross and histological

examination of tissues following autopsy has shown in the organs of zinced rats no damage of any sort attributable to zinc.

The normal amount of zinc excreted in the urine of rats is shown to range approximately between 0.02 and 0.20 mgm. per week; that in rat feces, between 1.25 and 7 mgm.

Our studies upon zinc excretion both in control and in zinc-fed rats indicate that a small fraction of absorbed ingested zinc leaves the body in the urine but that the main bulk of it is excreted through the gastro-intestinal tract. Highly zinced rats show increases over the normal in urinary zinc but these increases are relatively smaller than we have previously shown to be the case with highly zinced cats and dogs. It is worthy of note, nevertheless, that for months rats may excrete abnormal amounts of zinc through the kidneys without any clinical or histological evidence of kidney damage.

Figures are given upon total zinc ingested and total zinc excreted—figures which indicate that rats are able to excrete as much zinc as they ingest, even when the zinc dose is very large. This fact is true whether the ingested zinc be an organic zinc compound or the more insoluble zinc oxide.

Data are presented upon the zinc concentrations in the tissues and organs of control and of zinced rats. These data indicate that the ingestion by rats of zinc oxide or of organic zinc compounds over many months and even in large amounts does not significantly increase the zinc concentration in this species of animal—that is to say, rats do not tend to store zinc in amounts significantly above the normal but their excretion of this metal tends to keep pace with its ingestion.

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THE UTILIZATION OF DEXTROSE, LEVULOSE AND GALACTOSE BY ANIMAL AND PLANT CELLS, AND THE ANTAGONISTIC ACTION OF INSULIN TO THYROXIN

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The object of the present investigation was to determine the rate at which the three simple sugars, dextrose, levulose and galactose, are used by individual animal and plant cells, and the effect of insulin and thyroxin on this rate of utilization. The animal cells used were paramecium caudatum and the plant cells spirogyra porticalis. The paramecia were grown on an infusion made of horse manure, pond lily leaves and lake water at ordinary room temperature. The most difficult part of this investigation was the raising of these organisms in sufficiently large quantities and in fairly pure cultures. The spirogyra was collected from a nearby lake. Sugar determinations were made according to the method of Benedict.[1]

The paramecia were collected, centrifugalized and washed free of debris in a small centrifugalizing machine. The quantity of paramecia used in each of the experiments was approximately 5 cc. The centrifugalizing tubes were graduated in cubic centimeters so that the paramecia were measured as they were centrifugalized. In the work on spirogyra, 40 grams of this material were used in each of the experiments. After collecting the spirogyra, it was brought to the laboratory and the excess of water removed by gently squeezing it with the hands. It was then divided into batches of 40 grams each.

Fifteen cubic centimeters of paramecia were collected and introduced into 300 cc. of aerated lake water. This material, after being thoroughly mixed by pouring back and forth from one vessel to another, was divided into portions of 100 cc. each. In one portion 100 mgm. of dextrose were dissolved; in another 100 mgm. of levulose; and in the third, 100 mgm. of galactose. Sugar determinations were made immediately and subsequently at certain intervals. These three batches of paramecia sugar preparations were kept in a warm chamber at approximately 25°C., and

[1] We desire to thank Mr. J. D. McKinney of the Department of Chemistry for his help in making the sugar determinations.

air was bubbled slowly through the liquid to insure an adequate supply of oxygen to the organisms. The results of two typical experiments are given in figure 1, under *Paramecia*. It will be seen that the paramecia used all three sugars, and that dextrose and levulose were used more rapidly than galactose. In this respect the metabolism of sugar by these single celled animals is like that of the higher animals, for it is known that mammals use all three sugars, and dextrose and levulose are used more rapidly than galactose (1). It will be seen further in the chart that dextrose was used slightly more rapidly than levulose. In this respect the metabolism of the sugar by these single celled animals differs from what is claimed to be the case with the higher animals, for it is claimed that mammals utilize levulose

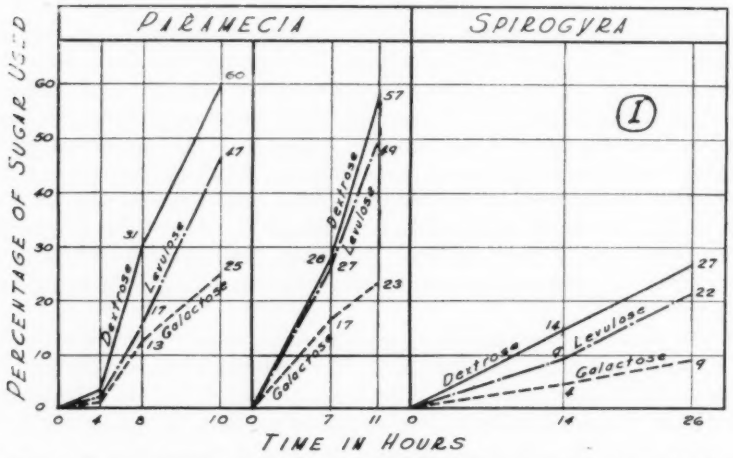


Fig. 1. Curves showing the comparative rate of utilization of dextrose, levulose and galactose by animal cells (paramecia) and plant cells (spirogyra).

more rapidly than dextrose (2). This conclusion is based on the fact that the respiratory quotient of mammals increases more rapidly when levulose is fed than when dextrose is fed. It is known that the three simple sugars after absorption from the alimentary tract may be converted into glycogen and stored in the liver and the muscles. However, when the glycogen is converted back into sugar it is always converted into dextrose and given off to the blood. So that the sugar normally presented to the tissues for use is dextrose and not levulose or galactose. Hence it would seem that if one sugar is more easily used than another, dextrose should be the one, as shown to be the case in this paper. Furthermore, it seems that where the cells are bathed in the sugar solutions and the sugar determinations are made directly as is done in the present experiments, it eliminates various

complicated factors that arise in determining the respiratory quotient in a mammal. In all, twelve experiments similar to the preceding were carried out with comparable results.

An objection that might be raised to the preceding experiments is that the bubbling of air through the solutions might, of itself, bring about a decrease in the amount of sugar. To meet this objection air was bubbled through sugar solutions not containing paramecia, and it was found that little or no change was produced in the sugar content. Another objection that might be raised is that bacteria in the water were responsible for the utilization of sugar noted. To meet this objection sugar solutions were made with the wash water from the paramecia after centrifugalization, and it was found that the amount of sugar used in these solutions was negligible; hence it would seem that the paramecia were responsible for the utilization of the sugar found in the preceding experiments.

We were next interested in determining the rate at which plant cells utilize the three simple sugars. One hundred milligrams of dextrose were dissolved in 100 cc. of lake water and in like manner solutions of levulose and galactose were made. Batches of spirogyra of 40 grams each were placed in these solutions and sugar determinations were made immediately and subsequently at certain intervals. The results of a typical experiment are shown in figure 1 under *Spirogyra*. It will be seen that spirogyra used all three of the sugars, just as was found to be the case with paramecium, but the rate of utilization was much slower than with paramecium. It will also be seen that spirogyra, as was found to be the case with paramecium, used dextrose and levulose more rapidly than the galactose, and the dextrose more rapidly than the levulose. Several experiments similar to the preceding have been carried out during the past three years with comparable results. Similar experiments with spirogyra were carried out in ordinary daylight and in the dark, and it was found that light had little or no effect on the rate of utilization of the sugars.

The following experiments were performed to determine the effect of insulin on the rate of utilization of sugar by these plant and animal cells. Thirty cubic centimeters of paramecia were collected and added to 600 cc. of lake water. After being thoroughly mixed, this material was divided into portions of 200 cc. each. Two hundred milligrams of dextrose were dissolved in one 200 cc., and 200 mgm. of levulose and galactose respectively dissolved in the two remaining 200 cc. portions. Each of these 200 cc. sugar-paramecia preparations were then divided into two parts of 100 cc. each. To one of these parts containing the dextrose, one vial of U 20 insulin was added. In like manner similar amounts of insulin were added to 100 cc. solutions of levulose and galactose. Amounts of water equal in volume to the amount of insulin (approximately 5 cc.) were added to each of the controls. Sugar determinations of the solution to which the

insulin was added, as well as the controls, were made immediately and subsequently at certain intervals. All of these materials were kept at approximately 25°C. during the experiments, and air was bubbled through the sugar solutions containing the paramecia at the rate of a bubble every few seconds. The results of a typical experiment are shown in figure 2

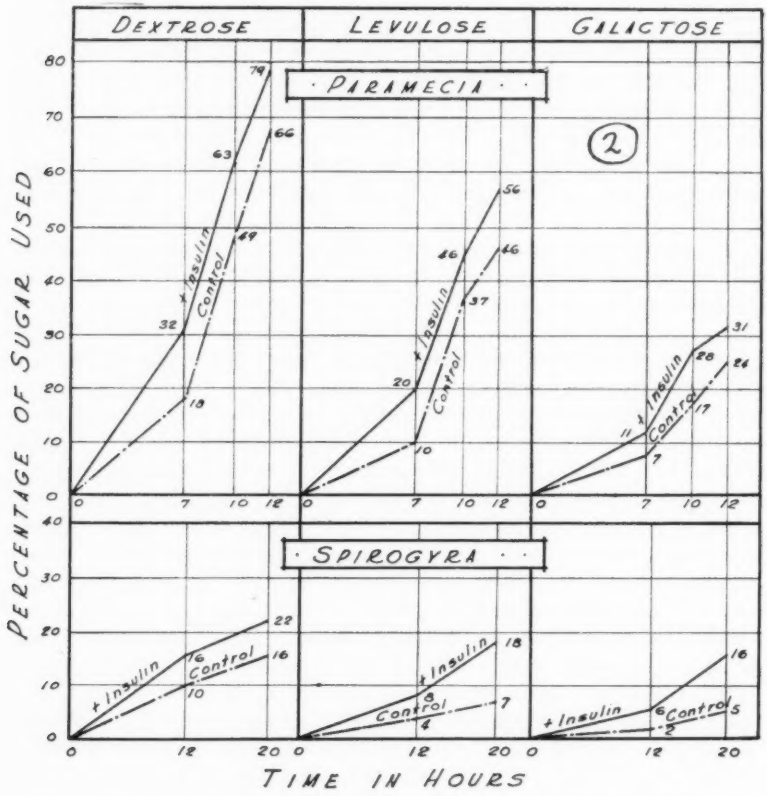


Fig. 2. Curves showing that insulin increases the rate of utilization of dextrose, levulose and galactose by animal cells (paramecia) and plant cells (spirogyra).

under *Paramecia*. It will be seen in this figure that the insulin increased the rate of utilization of all the sugars over the normal. In this respect these single celled animals resemble the higher animals, for it is known that the injection of insulin into the blood stream of a mammal greatly increases the rate of utilization of sugar (3). Experiments similar to the preceding with paramecia were carried out with varying amounts of insulin, and it

was found that smaller amounts of insulin gave smaller increase in the rate of utilization of the sugar. Nine experiments with the use of insulin were carried out with very similar results.

Two hundred milligrams of dextrose were dissolved in 200 cc. of lake water. Similarly solutions of levulose and galactose were made. Each of these was then divided into equal portions of 100 cc. each. One vial of U 20 insulin was added to each of the dextrose, levulose and galactose solutions, and water equal in volume to that of the insulin was added to each of the controls. Forty milligrams of spirogyra were then placed in each of these solutions, and sugar determinations were made immediately and subsequently at certain intervals. The results of a typical experiment are shown in figure 2 under *Spirogyra*. It will be seen that insulin increased the utilization of all three of these sugars by the spirogyra, just as was found to be the case with the paramecia. Five experiments similar to the preceding were carried out with comparable results.

The preceding experiments would seem to indicate close metabolism of the sugar by these lower organisms and the higher animals. Because of this resemblance and the comparative simplicity of the experiments with paramecia, it would seem desirable to test out the effect of various substances on sugar metabolism concerning the action of which in the higher animals there are at present contradictory results. Cushing (4), for instance, found the removal of the pituitary body increased sugar tolerance, whereas in acromegaly it was decreased. Forschbach and Severin (5), contrary to Cushing, found that there was hypoglycemia and increased sugar tolerance in acromegaly, as well as in other varied affections of the hypophysis. We have carried out preliminary experiments in which pituitrin was added to sugar solutions containing paramecia and found that the sugar metabolism was increased.

The following is a description of experiments showing the effect of thyroxin on the sugar metabolism of paramecium. Forty-five cubic centimeters of paramecia were collected, as described previously, and introduced into 900 cc. of aerated lake water. While being thoroughly mixed by pouring from one vessel to another, these 900 cc. of liquid were divided into three portions of 300 cc. each. To one portion, 300 mgm. of dextrose were added. To the two others, 300 mgm. of levulose and galactose respectively were added. While being thoroughly mixed, each of these 300 cc. batches were divided into three portions of 100 cc. each. Each 100 cc. portion was then introduced into a sedimentation glass and air was bubbled through the liquid at a slow rate. To one of the 100 cc. dextrose-paramecia preparations half a grain of thyroxin dissolved in 5 cc. of water was added. To another 100 cc. dextrose-paramecia preparation one 5 cc. vial of U 20 insulin was added; while to the remaining 100 cc. preparation, which served for control, 5 cc. of water were added. Similarly thyroxin,

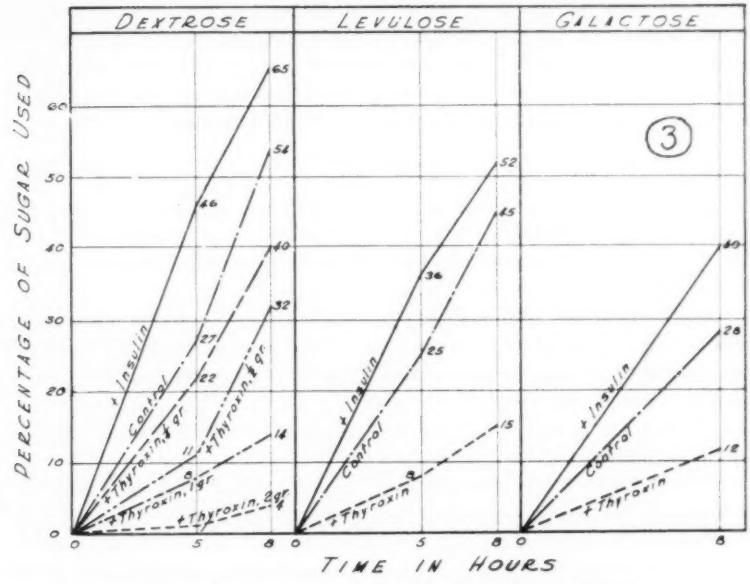
insulin and water were added to the levulose and galactose portions. Sugar determinations were made immediately and subsequently at intervals as shown in figure 3. It will be seen in this figure that the thyroxin decreased the utilization of all three sugars, and that insulin increased it. It will also be seen that varying amounts of thyroxin were used with the dextrose preparations, and that two grains of the thyroxin reduced the sugar metabolism to almost zero.

During the past year a number of experiments similar to the preceding have been carried out and it was invariably found that thyroxin decreased the sugar utilization, while the insulin increased it. Another thing to which we have never found an exception is that dextrose and levulose are always used more rapidly than galactose.

The next experiments to be described were carried out to determine the effect of a mixture of thyroxin and insulin on sugar utilization. Forty-five cubic centimeters of the organisms were collected, introduced into 900 cc. of lake water, and divided into portions of 300 cc. each. Dextrose, levulose and galactose were added, and each of the 300 cc. portions were divided into portions of 100 cc. each, as described in the preceding experiment. To one 100 cc. portion of the dextrose-paramecia preparations, $\frac{1}{2}$ grain of thyroxin dissolved in 5 cc. of water was added. To another 100 cc. portion, one 5 cc. vial of U 20 insulin was added. To the remaining 100 cc. portion, a mixture of $\frac{1}{2}$ grain of thyroxin and one 5 cc. vial of U 20 insulin was added. Sugar determinations were made immediately and at intervals, as shown in figure 4. It may be seen in this figure that the greatest utilization of sugar took place in the portions to which the insulin was added, the least in the portions to which the thyroxin was added, and an intermediate amount in the portions to which the mixture of insulin and thyroxin was added. This observation is interpreted to mean that thyroxin antagonizes the action of insulin.

The next question that interested us was: will the introduction of thyroxin into an animal counteract a subsequent injection of insulin. As yet this work has not been completed; but as far as time has permitted us to carry it, the indications would seem to be that it does. We have introduced large quantities of thyroxin (6 to 12 grains) into rabbits by means of stomach tubes, and 20 minutes later injected five units of insulin subcutaneously. We found that the effect of the thyroxin was to retard the development of the convulsions, even when the dose of insulin given to the thyroxin rabbit was twice as great as that given to the control.

It has been observed that there is a tendency to glycosuria and a lowering of the respiratory quotient in people with exophthalmic goiter, as well as in animals to which thyroxin has been administered. DuBois (6) attributes this glycosuria to an inability of the liver to store glycogen, while Cecil (7) attributes it to lesions of the pancreas resulting in a diminished



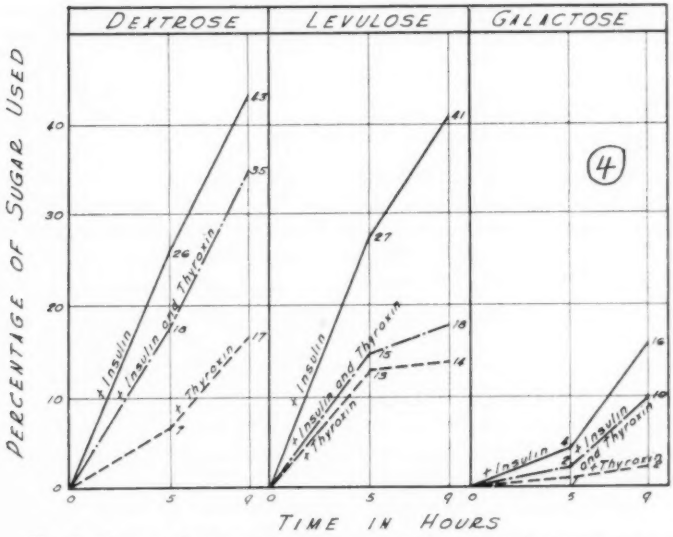


Fig. 3. Curves showing that thyroxin decreases and insulin increases sugar metabolism in paramecium caudatum.

Fig. 4. Curves showing the antagonistic action of thyroxin to insulin on sugar metabolism.

output of insulin just as is the case in diabetes. The experiments reported in this paper suggest that the lowered respiratory quotient and glycosuria in animals to which thyroxin has been given as well as in exophthalmic goiter may be due to a decrease in the activity of the insulin brought about by the antagonistic action of the excess of thyroxin.

SUMMARY

1. Animal cells (paramecia) and plant cells (spirogyra) utilize dextrose, levulose and galactose.

2. The animal cells utilize these sugars more rapidly than the plant cells.

3. Both the animal and the plant cells utilize dextrose and levulose more rapidly than the galactose, and the dextrose more rapidly than the levulose.

4. Insulin increases the rate of the utilization of the sugars, both by the plant and the animal cells.

5. Ordinary daylight produces little or no effect on the rate of utilization of the sugars by the plant cells.

6. Thyroxin decreases the utilization of dextrose, levulose and galactose in animal cells (paramecia), while insulin increases it.

7. Thyroxin antagonizes the action of insulin on sugar utilization, which may be responsible for the lowered respiratory quotient and glycosuria in exophthalmic goiter, as well as in animals to which thyroxin has been given.

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THE EXCRETION OF WATER BY THE KIDNEYS OF FROGS

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Studies of the activities of frogs' kidneys have until recently been concerned entirely with the allocation of functions among the kidneys' visibly differentiated structures. It has become evident, however, that the first essential is to know accurately how the whole kidneys perform in their intact condition. Some information of this sort is now available in the papers of Przylecki (1922), Przylecki, Opienska and Giedroyc (1922), Wearn and Richards (1924), DeHaan and Bakker (1924) and Ucko (1926).

In the observations to be described attention was focussed upon the excretion of *water* by normal frogs. After the variations in rate of excretion under standard conditions were evaluated, the influences of various simple changes of environment were measured. Finally, the question whether diuresis can occur in normal frogs was answered.

The manipulation to which the frogs (Rana pipiens) were subjected consisted in ligating the cloacas with coarse thread. After some practice this ligation could be rapidly performed, and was successful in the majority of instances. The success or failure of the ligation was not always to be judged by inspection, and we therefore used throughout our work the laborious method of weighing the frogs individually at intervals of about two hours, except in overnight periods. Upon plotting the changes of body weight against time, one could make certain whether or not the retention of urine was complete. The procedure of an experiment is illustrated in figure 1.

After a period which was usually either about six or about twenty-four hours, the cloaca was untied by pulling off the ligature with forceps. The urine was collected as it issued, and the frog was massaged to complete the expulsion. The measurement of the volume of this urine as collected was not, however, relied upon as a datum; instead each frog was weighed, with standard draining, just before and just after the ligature was removed. As a result of the tight ligation of a button of skin about the cloaca, the skin often sloughed or tore in the immediate region of the cloaca. Only in a small number of cases was more than one measurement done on the

same individual; and therefore it became necessary to treat the data statistically.

1. The normal rate of water excretion. The standard conditions which were taken as normal were as follows: Frogs were kept immersed in tap water at room temperature (18° to 23°C.) for at least two days, and usually longer, before measurements of urine were made. During the period of ligation and urine accumulation the tap water was not changed. That our tap water was not toxic in any respect was indicated by the fact that tadpoles and flatworms, which were readily killed by the ordinary tin-condensed distilled water, endured this medium indefinitely. Adult

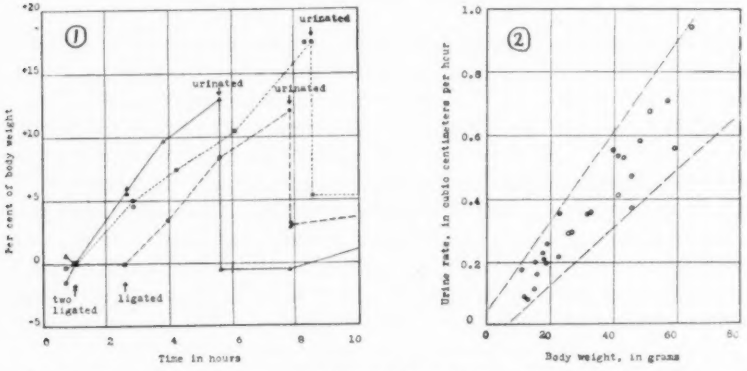

Fig. 1. Changes of body weight of frogs whose cloacas were ligated at the times indicated. The three individuals were transferred from tap water to 0.08 M NaCl at the time of 1 hour. The frogs urinated when the cloacal ligatures were removed, the subsequent weights indicating how much of the change of body weight was due to the urine accumulated in the bladder and cloaca.

Fig. 2. Correlation between the absolute rates of urine formation and the body weights of the individual frogs. All these frogs were immersed in tap water at 18° to 23° during the period of 5 to 28 hours in which the urine was made to accumulate in the bladder and cloaca.

frogs were never killed by either of these media. All the solutions used were made up in glass-condensed distilled water.

Figure 1 shows that for the individual animal the amount of urine which accumulated was nearly proportional to the time of its accumulation. This was found to be true for periods up to 48 hours. It was previously shown to be true for Rana esculenta at 14°C. by Przylecki (1922), and indicates that retention of urine in the bladder and cloaca does not interfere with the water-excreting activity of the kidneys.

The amount of urine excreted per hour was small for small individuals and greater for large individuals. Figure 2 shows all our measurements

upon frogs in tap water at 18° to 23°, relating the rates of urine excretion to the basal weights of the individuals. It is apparent, as has been already pointed out (Adolph, 1927) that on the average the amounts of water excreted per hour were directly proportional to the body weights. This correlation shows that we may compare individual results more profitably by recording the rates of urine excretion as percentages of the body volumes. It also indicates how much variation to expect between individuals, and that this variation will be relatively least in large frogs.

Such a correlation may precariously be taken to mean that the rate of urine formation was not a function of the body surface, but rather of the body mass. This suggests that the normal excretion of water was determined by the amount of kidney tissue, rather than by the area of skin exposed to a medium which was contributing fluid to the body continually (Adolph, 1927).

2. Influence of temperature. The variation of the rate of water excretion with temperature is shown in figure 3. The rate of formation was doubled or trebled for every increase in temperature of 10°C. It is also worthy of note that the variability was not only absolutely but also relatively greater at high temperatures than at low ones.

In order to obtain accurate data in these measurements, it was necessary to allow the frogs to adjust to the new temperature for about four hours before the period of urine accumulation began. This precaution was disregarded in the first series of determinations here represented.

At extremely low temperatures almost no urine could be found at the end of six or twenty hours. We finally obtained accurate results, however, over periods of ninety-six hours.

At low temperatures we found (Adolph, 1927) that the body weights of frogs increased markedly. When taken from 20° and placed at 1°, this increase amounted to about 8 per cent of the body weight. The change was usually completed in 24 hours. At first we supposed that at the low temperatures the regulation of water content was less precise; perhaps because the kidneys went into inactivity. But the rates of the water excretion turned out to be highly consistent and regular, and the body weights were at least as constant at 1°C. as they were at room temperature. The data showing increases in the water contents of various tissues of frogs in hibernation (Ott, 1924) therefore indicate new levels of water content, and not mere failures of regulation.

The influence of temperature upon the rates of urine formation in normal frogs has previously been measured by Overton (1904), Przylecki, Opienska and Giedroyc (1922), and DeHaan and Bakker (1924). In each case the data fall into a curve similar in shape and magnitude to the one in figure 3. All of the present measurements were made upon winter frogs, though most of the individuals had had no opportunity to hibernate.

3. Influence of the medium. It is well known that when placed in various concentrations of sodium chloride, frogs gain weight in some and lose weight in others. It is conceivable that the shifts in the balance of water content

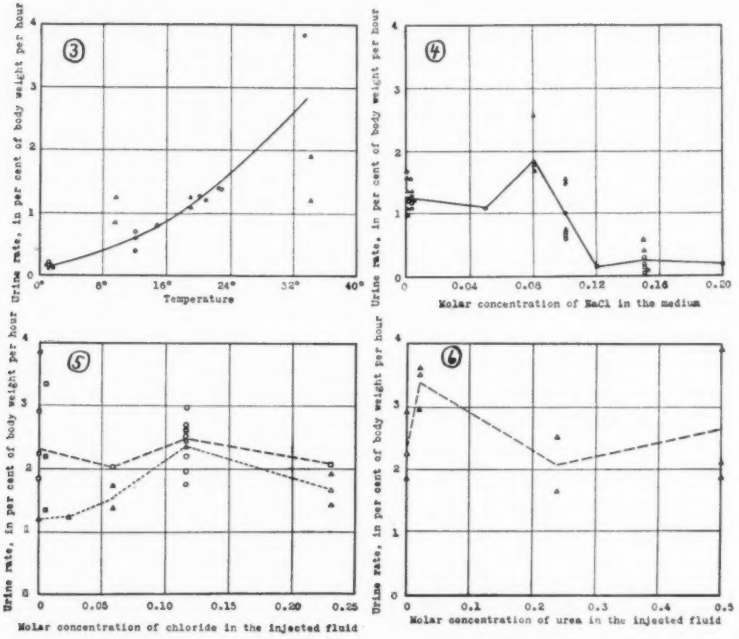

Fig. 3. Rates of urine formation of frogs kept in tap water at various temperatures. Triangles represent the first experiment, circles the second experiment.

Fig. 4. Rates of urine formation of frogs immersed in various solutions of sodium chloride. The three types of points represent the data obtained at three separate times.

Fig. 5. Rates of urine formation of frogs just after they were injected with salt solutions of various concentration. The salts of Ringer's solution were present in the same relative proportions throughout. Triangles represent the first experiment (24 hours of urine formation), crossed circles the same with 5 per cent of acacia added. Squares represent the second experiment (6 hours of urine formation), open circles the same with one-fourth or one-eighth per cent of caffein added.

Fig. 6. Rates of urine formation of frogs just after they were injected with urea solutions of various concentrations. All collections of urine represented six-hour periods.

may be due at least in part to changes in the rate of water excretion. To determine this point we carried out three separate series of measurements. In the very first of them it became evident that in solutions which were hypertonic to frog's blood the rate of urine formation was greatly decreased.

Nevertheless, it appeared that in concentrations up to 0.10 M NaCl the rate of excretion was uniform. It is just in this range of concentrations that frogs gain in net weight, and it seemed important to determine more accurately whether the kidneys really increase or decrease their activity with respect to water. In the second series it appeared that at one concentration (0.08M) the rate was uniformly higher than in tap water, and in at least one frog significantly so. In a third series this was not confirmed, nor was a diminution in the rate of excretion, such as Overton (1904) found in his single measurement, demonstrated for this range of concentrations. We have to conclude for the time being, therefore, that the rate of water excretion is not greatly modified by changes in the concentration of the medium up to 0.10 M NaCl.

The influence of solutes in the medium other than NaCl was not studied systematically.

When frogs were removed from water and placed in a moist atmosphere they ceased altogether to form urine. When the individuals which had been out of water for a day or two, and which had lost some weight by evaporation, were replaced in water, they immediately formed urine at the normal rates, in addition to making up for the water lost.

In another experiment some frogs were placed in a vessel containing only a shallow layer of water while their urine rates were measured. In the course of twenty-four hours the water was twice completely absorbed from the vessel by the frogs. Yet urine was found in their bladders to the normal extent. The frogs had each gained in weight by the amount of urine accumulated, but had not changed from their basal values in net weights, and did not gain at all in net weight, when re-immersed in water.

It is clear that in general the kidneys were not influenced by the medium as long as water was being absorbed through the skin at the usual rate. Only when water was denied, so that the net weight of the body began to diminish, was urine formation inhibited.

4. Injection of fluids. Having evaluated the influences of several factors external to the body, we attempted to modify the internal environment so as to stimulate the kidneys in respect to water excretion. Various fluids were injected under the skin into the dorsal lymph sacs. The amounts injected varied up to 25 per cent of the body weight; but not all of the fluid was retained, for the puncture in the skin allowed some to escape. The amounts actually kept under the skin were ascertained by following the weights of the individual.

The normal rates of urine formation in tap water averaged 1.3 per cent per hour, and never exceeded 1.7 per cent. But after injection many of the rates exceeded 2.0 per cent, and frequently were double the average rates. That the skin absorbs water at the standard rate after an injection has been made, is apparent from the time courses of increase in body

weight; whence it is to be expected that the urinary water excretion is increased in rate.

In figure 5 are shown the urine rates of frogs which were injected with distilled water, with tap water, or with balanced salt solutions of various concentrations. Significant increases in urine rate resulted from all the injections tried. With Ringer's solution of double the ordinary concentration, however, the augmentation of water excretion was less marked than with more dilute fluids. In figure 6 results are shown after injecting various concentrations of urea.

In the earliest of these measurements the urine was allowed to accumulate over-night before collecting it. From these results we gained the impression that no diuresis had been produced by the injections. It was then found, however, that over short periods of time the augmentation was so marked that no dispute could arise as to its being a true diuresis. For, at an average rate of 1.3 per cent of its body weight per hour, a frog would normally excrete 31 per cent of its weight in 24 hours. While such an amount of fluid was being excreted, an additional amount equivalent to only 10 per cent of the body weight would naturally appear inconsequential, or even doubtful, because within the limits of variation under standard conditions. But an amount equivalent to 10 per cent of the body weight when added to the normal rate for six-hour periods more than doubled the excretory rate. And as a matter of fact it was found that practically all the injected fluid left the body within six hours in the case of water and of urea solutions.

Figure 7 indicates the influence of the *amount* of injection upon the urine rate; heretofore this factor was not taken into account. In general, then, the diuretic effect was larger the more fluid there was requiring elimination, and the shorter the period after the injection during which the urine was collected.

It may also be observed in figure 7 that the injection of Ringer's solution gave much less diuresis than the same quantity of water or of a urea solution. This failure to excrete promptly an isotonic solution consisting largely of sodium chloride was found to be characteristic in the case of man (Adolph, 1923). In addition to solutions of balanced salts and of urea, the effect of a so-called diuretic drug, caffein, was tested. Using 0.25 per cent solutions, either in water or in Ringer, some of the animals were killed. But half this concentration proved harmless. The values obtained after caffein injections are shown in figure 5; no difference can be noted as compared with the values after injections of the corresponding fluids without the drug.

In a few cases the effect of adding 5 per cent of acacia to the Ringer's solution was tried. As shown in figure 5, this made no sensible difference as compared with Ringer alone.

5. The concentration of frogs' urine. The urine excreted by frogs under standard conditions was extremely dilute. This was originally shown by Overton (1904) and by Toda and Taguchi (1913). We found that frogs which have been fed and active, average a total concentration, as measured by the freezing point, of 0.05 osmolar. Starved frogs tended to fall below this figure, and excreted practically no chloride.

When placed at various temperatures frogs produced urines which were, on the average, more dilute in the cold and more concentrated in the warm. This has been shown by Overton (1904) and by Van der Heyde (1921) to

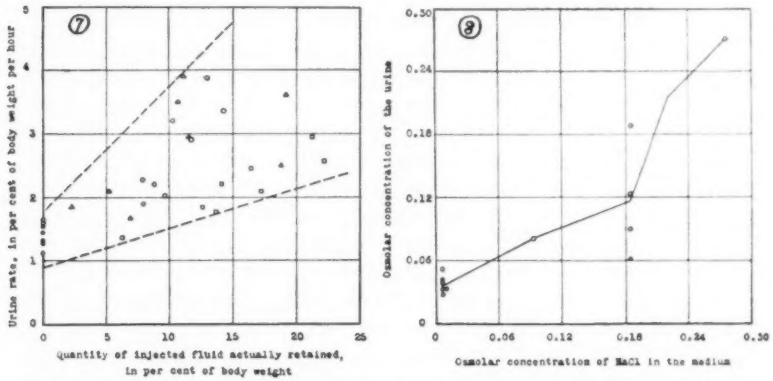

Fig. 7. Rates of urine formation of frogs in relation to the amount of injected fluid which was actually retained under the skins. All collections lasted for three to seven hours. All of the data of figure 6 and half of the data of figure 5 are here reproduced. Circles represent injections of distilled or tap water, squares salt solutions, and triangles urea solutions.

Fig. 8. Total concentrations of the urines of frogs while they were immersed in sodium chloride solutions of various concentrations. In concentrations greater than 0.12 M (0.22 osmolar) the urinary concentrations are equivalent to those of the medium.

be true as well for the nitrogenous and pigmentary constituents of the urine.

When placed in various concentrations of sodium chloride the total concentration tended to increase, as shown in figure 8. The increase was in part but not wholly due to the excretion of more chloride. In 0.15 M NaCl the total concentration of the urine was equivalent to that of the medium. Brunacci (1912) showed that this was true in media of approximately this concentration not only for the urine, but that the blood and the lymph also attained the same concentration. We have found that the equality of concentrations in frogs was attained by a loss of water from the body through the skin (Adolph, 1927).

When injected with various concentrations of Ringer's solution, the urine excreted had a total concentration which was correlated with the concentration of the injected fluid. In the case of 0.113 M Ringer the urine was 0.075 osmolar, and in the case of 0.226 M Ringer the urine averaged 0.149 osmolar. Przylecki (1922) found that with more highly concentrated injecta, in frogs which were kept out of water, the urine might become concentrated up to 0.48 osmolar, but that the blood was always slightly more concentrated than the urine.

No instance has been recorded where the urine of a frog was more concentrated than the blood plasma at the time. The same rule has been found to hold for other amphibia and all fishes (Burian, 1910).

It is clear, therefore, that while in man the maximal concentration attained by the urine may be four times the concentration of the blood (Adolph, 1923), and in the cat eight or ten times the concentration of the blood, the urine of frogs has its maximal concentration set at equality with the blood. Since a hypertonic condition either outside the frog or in the frog's lymph spaces inhibits urine formation until osmotic balance has been established between tissues, lymph, blood and urine, it would be only by a most ingenious trick that one could produce in a frog the conditions required for "salt diuresis," i.e., excessive excretion of water due to the necessity for eliminating solutes. Kunde (1857) described the rapid loss of weight by frogs when crystals of salt were introduced under the skin. Whether this loss was through the kidneys it would be premature to state, and that his observations represent a case of salt diuresis would be far from probable.

Individual constituents may be and regularly are concentrated by the frogs' kidneys. Przylecki (1922) and DeHaan and Bakker (1924) have demonstrated this activity with respect to urea upon frogs under normal conditions.

6. The regulation of water excretion. The rarity with which diuresis occurs in frogs might be expected from the rapid rate at which water is normally excreted by frogs. While a man on the average excretes one-fiftieth of his weight of water per day, a frog excretes one-third of his weight per day. The maximal rates of excretion for man, even over short periods of time, barely rival the frogs' normal performance (Haldane and Priestley, 1916). Under the most drastic conditions that injections imposed, frogs barely excreted urine at the rate of the body weight per day over a period of a few hours. This in turn was a modest feat compared with the normal turn-over of water in certain protozoa (Adolph, 1926).

Which organs are concerned in this turn-over of water in frogs is now quite clear. The water enters the body through the skin at a rather constant rate. This rate is evidently not controlled by each unit of skin area, for if only a small portion of the skin is in contact with water, this

takes up as much as the whole skin regularly would. If the medium contains solutes, water is still taken up at the normal rate, as long as the medium is more dilute than the blood. The kidneys excrete this water at the same uniform rate, so that the net volume and net concentration of the body are constant. When the supply of water from the medium fails, or when the supply is augmented by injections, then they modify their activities somewhat.

Certain experiments of Przylecki (1922) seem to indicate that the kidneys are not very flexible in their adjustment to bodily needs. He reported some results on frogs whose ureters were ligated, but did not describe how the ligation was performed. When ligated bilaterally, the body weight after a time ceased to augment; evidently the skin had responded to the interruption of water metabolism. But when only one ureter was ligated, then water was excreted at only half the rate that it was when both kidneys were functioning. This inflexibility of the kidneys under ordinary conditions is demonstrated by the constancy with which they produce urine which is extremely hypotonic. As far as water is concerned, their ordinary activity provides for nearly all contingencies; in fact, for all contingencies except when water is injected subcutaneously. Whether even then the water-excreting function is in part taken over vicariously by the skin, has not been ascertained; our data appear to show that excessive water injected under the skin is all accounted for in the subsequent urine.

It is evident in the many experiments which have been done by various investigators with frogs' kidneys under operative and perfusion conditions, that the urine then produced is grossly abnormal in composition. In all, the chloride concentration, which as we have shown is normally 0.01 M or less, tends to become equal to that of the perfusing fluid. It appears unprofitable to compare conclusions reached under such conditions with the results obtained upon normal frogs with the cloacas ligated.

Mr. C. C. Smith assisted in the work here described, and his help is gratefully acknowledged.

SUMMARY

1. The average normal rate at which frogs excreted water, when immersed in tap water at 20°C., was 1.3 per cent of their weight per hour.

2. The influence of temperature and the influence of the medium upon this rate were evaluated.

3. Diuresis was produced in frogs by injecting water or solutions of urea or of chlorides under the skin.

4. The urine of frogs was ordinarily very dilute. It was never hypertonic to the blood plasma.

5. The kidneys of frogs failed to show under some circumstances any adaptation to the requirements of the organism.

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THE EFFECT OF THE FOLLICULAR HORMONE ON OLD ALBINO RATS[1]

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In the past few years a large number of articles bearing on various aspects of the sex hormones have appeared in the public press and in the scientific journals. The most sensational is that of rejuvenation by testicular transplants. That serious scientific investigators have striven to ascertain the true effect of the sex hormones is evidenced by the vast amount of literature which has recently appeared on this subject. Owing to this great accumulation it is unwise to embody in a short article a complete review. Only a few articles, therefore, will be mentioned.

It has long been known that the sex glands play an important part in the development of secondary sexual characteristics of the animal. This is shown by the fact that if the gonads are removed from a young immature animal there follows a failure of development of the secondary sexual characters common to it. There results also, as shown by Wang, Richter and Guttmacher (1925) and Hoskins (1925) and verified by us that there is a pronounced reduction of voluntary activity by the animal after gonadectomy. We have also noticed a marked tendency toward accumulation of adipose tissue accompanied by a reduction in the food consumption in animals deprived of their sex glands.

The sex hormone acts differently in the two sexes in its effect on spontaneous activity. This has been demonstrated by Wang (1923), Slonaker (1924), Durrant (1925) and others. In the female rat, during sexual life, it causes marked increases in voluntary activity which correspond with and are true indications of oestrus. This rhythmicity in activity is wanting in ovariectomized rats, in females before pubescence, after the menopause, and usually during pregnancy and the first two or three weeks of lactation. This rhythmic activity is not found in the normal male. Wang and co-

[1] This research has been conducted with the aid of the Department of Physiology and the Research Fund of Stanford University, and the Committee for Research on Sex Problems of the National Research Council. I wish also to acknowledge with thanks the assistance of Dr. Edgar Allen of the University of Missouri for sending me hormone prepared by him and the aid of Mr. J. O. Snyder, Manager of the Western Meat Company, San Francisco, for generous supplies of pig ovaries.

workers (1925) have, however, induced spontaneous rhythmic activity, corresponding to true oestrual rhythm of the female, in castrated males by successful transplantation of functioning ovaries. This rhythm in activity ceased when the implanted ovaries were removed and the activity dropped to the low level characteristic of castrated males. The transplantation of the testes either as a whole or as a graft, or the injection of testicular substance into ovariectomized females, castrated males, or in old or degenerate animals by different investigators has been followed by varying results. (Lipschütz, 1923a, 1924b, 1926c; Arland, 1926a, 1927b; Moore, 1921; 1926b; and others.) Some have obtained successful transplants, but the general opinion is that the transplants sooner or later degenerate and the beneficial results, if any, are at best only transitory.

That the female sex hormone is closely associated with the developing ova and that it is most abundant in the follicular fluid of the ripe graafian follicles has been demonstrated by Allen and co-workers (1923a, 1924b), Frank and co-workers (1922a, 1925b, 1925c) and others. The active principle of this hormone has also been recovered from other fluids and organs of the body (Allen and co-workers, 1925c). These investigators have produced true oestrual changes in the vaginal mucosa of ovariectomized animals by subcutaneous injections of follicular fluid from ripe graafian follicules. They have also caused precocious sexual development by such injections in young females. These effects were determined by vaginal smears.

That at least some of the endocrine glands are closely related and interdependent is shown by the recent research of Smith (1926) in which he hastened the development of the female genital system by daily homoplastic pituitary transplants. By making daily transplants of the anterior pituitary he induced changes characteristic of sexual maturity in all the parts of the genital system, such as opening of the vagina, uterine hyperemia and distention, follicle and corpora formation and vaginal smears of the oestrual type, in rats as early as 22 days of age. He apparently did not try the crucial test of sexual maturity by mating the animals. When the transplants were begun on animals twenty-two days old it took five or six days to reach sexual maturity; when begun at the age of fourteen days it took eight to ten days. A single transplant was unsuccessful. Daily transplants in adult females caused an increase in the size of some of the follicles, which often became encysted, and the ovaries hypertrophied reaching a weight from 2 to 4 times that of the normal control. Since he was unable to get similar results on ovariectomized rats he concluded that the pituitary transplants, per se, did not cause the precocious sexual development, but that the early maturing was due to the ovarian (follicular) growth induced by the transplants. Marinus (1919) has shown that the

feeding of the anterior lobe of the pituitary body caused earlier sexual development than normal.

The above results of the ovarian hormone were all obtained from relatively young or growing animals. The purpose of these experiments was to determine, if possible, the effect of the ovarian hormone on older females and those which had passed the menopause in regard to the vaginal smear, vaginal opening, mating, reproduction, food consumption, body weight and spontaneous activity. The animals used consisted of normal female rats, some which had been ovariectomized, and some others which had been hysterectomized but in other respects were normal. The ages varied between 538 days to 971 days. It was hoped that some information might be gained whereby the productive sexual life of a valuable breeding animal might be prolonged or the often direful results of the more or less sudden cessation of sexual activities at the menopause might be alleviated.

The hormone used was of two kinds, fresh and preserved. The fresh was obtained by aspirating the follicular fluid from the large follicles of the fresh pig ovaries. The ovaries were packed in ice on removal from the pigs and transported at once to the laboratory and aspirated. The subcutaneous injections were begun usually within three or four hours after the death of the donor. The second source of the hormone was from Doctor Allen (quoted above) who kindly furnished me with two supplies which he had tested and found potent. The number of injections given each animal varied from one to nine. Never more than three injections were given in a day and these were at intervals of approximately four hours. Daily observations were made and tabulated of the smears, vagina, mating, food consumption, body weight and spontaneous activity.

Four experiments were performed and these will be given in order. The results of each experiment have been put in tabular form. The + and − signs indicate either positive or negative results, or an increase or decrease in the food consumption, body weight or voluntary activity. The source of the hormone used is indicated by P (fresh from pig) and A (from Doctor Allen). The voluntary activity was ascertained by revolving cages. The animals whose records indicate changes in activity had been in revolving cages for months and accustomed to turning them.

Experiment 1. The hormone used was secured from pig ovaries and was injected subcutaneously into the rats in fresh undiluted condition within three or four hours after removal from the donor. Only one injection was given each animal but the amount used varied from 0.5 cc. to 1.5 cc. per dose. Eight rats, four normal and four ovariectomized, were used. The age of normal animals ranged between 525 and 785 days while that of the ovariectomized was 525 days. The results have been tabulated in table 1. By consulting this table it is seen that the normal animals gave variable

results and that in the ovariectomized rats they were more uniform. The vaginal smears showed that all the normal rats were still running their oestrual cycles. The positive smear tests were therefore expected. The two cases of mating, both of which resulted in pseudo-pregnancy, occurred at a regular oestrus. While the majority of all the animals seem to indicate a decrease in food intake and in activity and an increase in body weight, the results are too meager for general conclusions. One is led to conclude that one injection of the hormone in varying doses is insufficient to cause an effect on ovariectomized rats. This is in accordance with results of other investigators.

Experiment 2. The hormone used in this experiment was from the pig

TABLE 1

Results of experiment 1

P, fresh pig hormone; c and o, closed and open; + and −, positive and negative, or increase and decrease.

RAT	NORM. HYST. OVCT.	AGE	NUMBER OF LITTERS	AGE AT LAST LITTER	AGE AT LAST OESTRUS	PRELIMINARY TESTS		HORMONE			RESULTS						
						Smears	Mating	Source	Number of injections	Cubic centimeters per injection	Smears	Days after first injection	Vagina	Mating	Food	Body weight	Activity
B5	N	525	3	479	524			P	1	0.75	+	0	o	+	+	+	−
F3	N	785	2	280	772			P	1	1.25	+	1	o	−	−	+	−
F5	N	780	5	549	776			P	1	1	+	1	o	+	−	+	−
E1	O	525	0					P	1	0.5	−		c	−	−	+	+
E2	O	525	0					P	1	1	−		c	−	−	−	+
E4	O	525	0					P	1	1.5	−		c	−	+	−	−
E5	O	525	0					P	1	1.25	−		c	−	+	−	−
D53	N	615	11	426				P	1	1.5	+	1				+	

ovary and was administered in the fresh and undiluted condition. Two or three injections of 1 cc. each were given. Two doses were given each animal at approximately 3 o'clock and 9 o'clock p. m. of the day the fluid was gotten. When a third dose was administered it was given early the following morning. The fluid was kept on ice when not being used. The results are condensed in table 2.

The animals used in this experiment were 20 normal (ages 596 to 874 days), 4 ovariectomized (ages 603 days), and 3 hysterectomized rats (age 633 days). Two of the normal rats (C2 and C5) were pregnant at the time of the injections and delivered young 18 and 4 days later respectively. The gestation period in each case was 23 days. Since this is the normal period

TABLE 2

Results of experiment 2

P, fresh pig hormone; c and o, closed and open; + and −, positive and negative, or increase and decrease.

RAT	NORM. HYST. OVCT.	AGE	NUMBER OF LITTERS	AGE AT LAST LITTER	AGE AT LAST OESTRUS	PRELIMINARY TESTS		HORMONE			RESULTS							
						Smears	Mating	Source	Number of injections	Cubic centimeters per injection	Smears	Days after first injection	Vagina	Mating	Food	Body weight	Activity
C1	N	611	5	405	610	+	−	P	2	1	∓	4		∓	+	+	+
2	N	611	5	521	606			P	2	1	−			−	+	+	−
3	N	611	6	458	611	+		P	2	1	+	0	o	+	−	+	−
4	N	611	8	595	610	+	+	P	2	1	+	0		−	−	+	−
5	N	611	6	458	592			P	2	1	−	5	c	−	+	+	−
D1	N	611	3	327	611	+		P	2	1	+	0		+	+	+	−
2	N	617	6	335	613			P	2	1	+?	2		+	+	+	−
3	N	596	9	588	596	+		P	2	1	+	0	o	+	−	+	+
4	N	596	7	324	594	+		P	2	1	+	0		+	+	−	−
5	N	596	8	525	583	+		P	2	1	+	0	o	+	+	+	−
C9	N	705	10	604				P	2	1	+	2	o	+		−	
10	N	705	12	685				P	2	1	+	0				+	
51	N	705	12	623		+	+	P	2	1	+	0		+		+	
52	N	702	12	651				P	2	1	+?	6	c			±	
D53	N	705	11	426		+		P	2	1	+	0		−		−	
54	N	702	11	408		+		P	2	1	+	0		+			
69	N	705	11	457				P	2	1	+?	9	c			+	
70	N	705	14	531				P	2	1	+?	11	c			+	
F2	N	874	7	520	874	+	−	P	3	1	+	0		+	+	+	−
5	N	869	5	549	863	+	−	P	3	1	+	0	o	+	+	+	−
E1	O	603	0					P	3	1	−		−	−	+	+	−
3	O	603	0					P	2	1	−		−	−	+	+	+
4	O	603	0					P	3	1	−?		−	−	+	+	−
5	O	603	0					P	2	1					−	+	
A6	H	633	0			+		P	2	1	+	0		−	±	+	−
7	H	633	0					P	2	1	+	0		−	+	+	−
8	H	633	0					P	2	1	+	2	o	+		−	

for rats of this age two injections of the hormone apparently did not interfere with normal development either in the early or late stages. Since smears, preliminary mating tests and activity curves indicate that all the normal rats were still running their cycles no conclusions can be drawn relative to the effect of two injections. The regular cycles did not seem to be interfered with in any way. The positive tests in the smears (table 2) in nearly all cases correspond with oestrual conditions found at the time of the first injections. In C52, D69 and D70 the smears were not positive until 6, 9 and 11 days respectively after the injections. It is very probable in these cases that the injected hormone was not instrumental in causing the vaginal changes, since they continued to appear at intervals for several weeks. The oldest normals, F2 and F5, copulated several times and showed a number of cycles during the six weeks following the injections. It is doubtful if the effect, if any, would extend over so long a time. All the copulations failed to result in pregnancy.

In the four ovariectomized rats used oestrus or mating was not induced by two or three injections. The three hysterectomized animals, whose activity curves had shown a complete absence of oestrus, gave positive smear but negative mating tests following the injection of two doses. The food consumption and body weight were increased and the voluntary activity decreased in the majority of cases. This decrease in activity is the reverse to that exhibited during normal oestrus and has not at present been explained.

Experiment 3. Hormone no. 12, which was furnished by Doctor Allen, was used in this experiment. He had found this hormone effective on injecting three 1 cc. doses of a mixture of 3 parts hormone 12 to 7 parts normal salt solution. He also found that mating would occur the evening of the second day or the morning of the third if three additional 1 cc. doses were given the second day. Using the hormone as suggested we injected from 3 to 9 doses of 1 cc. each. Three injections were given the first day and additional ones on successive days. Eight normal rats (ages 707 to 971 days), two ovariectomized (age 719 days), and two hysterectomized animals (731 days of age) were used. Preliminary smears, mating tests and observations were made daily on these animals for the eight days prior to beginning the injections. The results are given in table 3.

The preliminary tests showed oestrual conditions in three of the normal rats and in one hysterectomized. These conditions, however, were not accompanied by mating, which was negative in all cases. After the hormone injections, as indicated in table 3, two of the animals which showed positive preliminary smears mated. These two animals showed positive tests the morning of the first injection. This mating occurred at a regular oestrual period which seems to have been accentuated by the additional hormone. All other mating tests were negative. The smears had in-

creased from one-third positive to three-fourths positive and included the two ovariectomized rats which had previously failed to respond. These results seem to indicate that a certain amount of hormone will cause oestrual changes in the vaginal mucosa, and that a greater amount is neccessary to stimulate mating activities. The majority showed an increase in body weight and a decrease in activity and food consumption.

Experiment 4. The hormone used in this experiment was furnished by Doctor Allen and was designated P27. He had found it potent in the proportion of 3 parts hormone solution to 7 parts normal salt solution.

TABLE 3

Results of experiment 3

A 12, Doctor Allen hormone no. 12; c and o, closed and open; + and − positive and negative, or increase and decrease.

RA.	NORM. HYST. OVCT.	AGE	NUMBER OF LITTERS	AGE AT LAST LITTER	AGE AT LAST OESTRUS	PRELIMINARY TESTS		HORMONE			RESULTS						
						Smears	Mating	Source	Number of injections	Cubic centimeters per injection	Smears	Days after first injection	Vagina	Mating	Food	Body weight	Activity
B2	N	712	2	284	708	+	−	A12	3	1	+	0	o	+	−	−	−
C2	N	707	6	629	703	−	−	A12	6	1	+?	1	c	−	−	+	−
E1	O	719	0			−	−	A12	9	1	+	2	c	−	−	+	−
E4	O	719	0			−	−	A12	9	1	+?	2	c	−	−	+	−
F5	N	971	5	549	967	+	−	A12	3	1	+	0	o	+	−	+	−
At 2	H	731	0		698			A12	6	1	+	1	c	−	−	+	−
At 6	H	731	0			+	−	A12	6	1	+	1	c	−	+	+	+
D69	N	808	11	457				A12	6	1	−	8	c	−		+	
D54	N	805	11	408		+	−	A12	6	1	+	0	c	−		−	
C9	N	808	10	604		−	−	A12	6	1	−		c	−		−	
C51	N	808	10	623		−	−	A12	6	1	−		c	−		±	
C52	N	805	12	651		−	−	A12	6	1	+?	0	c	−		+	

The same proportions were used in our injections and the routine followed as described in experiment 3. Five 1 cc. doses of P27 were given each animal. These were followed in regular order of time by two additional injections of 1 cc. and 0.7 cc. each of a commercial hormone which we have called x. Preliminary smear and mating tests daily for the twelve days preceding the beginning of injections were made. The results are shown in table 4. Mating tests had also been made on the C's and D's for the preceding 77 days.

Ten normal females, approximately 850 days of age, were used. Each of these rats had delivered two or more litters and had always been active

breeders. All but one showed either positive or questionable cornified cells in the preliminary smears. During these twelve days the act of

TABLE 4

Results of experiment 4

AP 27, Doctor Allen hormone P 27′; c and o, closed and open; + and −, positive and negative, or increase and decrease.

						PRELIMINARY TESTS		HORMONE			RESULTS						
RAT	NORM. HYST. OVCT.	AGE	NUMBER OF LITTERS	AGE AT LAST LITTER	AGE AT LAST OESTRUS	Smears	Mating	Source	Number of injections	Cubic centimeters per injection	Smears	Days after first injection	Vagina	Mating	Food	Body weight	Activity
B2	N	850	2	284	783	+	−	A	5	1	+	3	c	−	−	−?	−
								x	2	1+0.7							
B3	N	850	3	473	783	+?	+?	A	5	1	+?	2	c	−	∓	−	∓
								x	2	1+0.7							
B4	N	850	3	477	829	+	+?	A	5	1	+	0	o	+?	−	−	−
								x	2	1+0.7							
B5	N	850	3	479	828	+	−?	A	5	1	+?	1	c	−	−	−	−
								x	2	1+0.7							
C2	N	845	6	629	828	+?	−	A	5	1	+?	7	c	−	+	−	−
								x	2	1+0.7							
C3	N	845	6	458	840	+	+?	A	5	1	+	3	c	+?	−	−	±
								x	2	1+0.7							
C4	N	845	8	595	828	+?	+?	A	5	1	+	2	c	−	−	−	−
								x	2	1+0.7							
D3	N	842	10	623	794	−	−	A	5	1	+	3	c	−	−	∓	−
								x	2	1+0.7							
D4	N	842	7	324	839	+	+?	A	5	1	+?	2	o	−	−	−	−
								x	2	1+0.7							
D5	N	842	8	525	826	+	+?	A	5	1	−	12	c	−	−	−	∓
								x	2	1+0.7							

coitus occurred in six cases but as spermatozoa were not demonstrated afterwards in the smears apparently true copulation had not taken place.

During the preceding 77 days the act of coitus occurred in C2, C3 and D4,

2, 7 and 7 times respectively. None of these cases resulted in pregnancy and only indicate that oestrus had taken place. The B's were tested only during the 12–day preliminary period.

After the injections of the hormone the smears showed positive or questionable results in all but one and the act of coitus in two. The latter were unsuccessful as shown by lack of spermatozoa in the smears. Observations were continued 12 days following the last injection and during this time but one attempted coitus occurred which was unsuccessful. Table 4 gives the number of days after the beginning of the injections that cornified cells appeared in the smears. Rats C2 and D5 showed cornified cells in the smears 7 and 12 days respectively after the beginning of the injections. In these cases it is doubtful if the added hormone had any effect.

The majority of the animals showed a decrease in food intake, body weight and voluntary activity. This agrees with the results of experiment 3 except in the effect on body weight. The only cause we can think of for this difference was the commercial hormone which was used in the last two injections. The conditions were the same in other respects except the animals averaged to be a little older. These conditions are but transitory and usually return to normal in a short time. The decrease in food consumption that was usually found was not great. The increased body weight associated with a decreased activity is what we would expect. The food energy which went into activity was stored and increased the body weight.

No attempts were made to determine whether ovulation occurred when cornified cells were found in old animals. The fact that not one of all the mating acts in old animals resulted in gestation and birth when sperm had been found in the smears would indicate that ovulation had not taken place. If this were true it appears that the presence of cornified cells in the smears of old females is not a true indication of oestrus and that their presence may be due, in part at least, to the action of some other endocrine gland or possibly to partially developed ova. A microscopic study of old ovaries would give us some information. In the old rats we found many cases where the vaginal smears indicated oestrus but only occasionally could we get even an attempt at mating. When the hormone injections almost coincided with that of the oestrual smear mating acts usually followed. That is, a smaller amount of the endocrine substance may cause the changes in the vaginal mucosa in old rats and yet be insufficient to incite mating activities. The additional hormone given in the injections in some may provide the stimulus which resulted in the consummation of mating.

As previously stated, there is exhibited in normal females during their sexual life a marked increase in voluntary activity at oestrus. This

increase is immediately followed by a sharp drop to a relatively low level. The cause of these changes has been attributed to the follicular fluid. It is very likely that ovulation occurs just before or at the peak of activity. If this assumption is true it appears that the liberation of the follicular fluid and its more ready absorption and distribution by the blood causes the sudden drop in activity. The injection of the hormone and its ready absorption and distribution would thus closely resemble normal conditions and the almost universal decrease of activity in our injected rats be explained.

SUMMARY

1. Cornified cells were found in vaginal smears long after the donors had passed the menopause, or had ceased to show marked increases in voluntary activity and mating activities. These cells often persisted for from one to three or four days.

2. It appears that oestrual changes in the vaginal mucosa can be incited by a smaller dose of the hormone than that required to excite mating activities.

3. One or two injections of fresh follicular hormone had little or no effect in modifying oestrual rhythm, or inducing mating in rats nearing the menopause. In older animals copulation may occur if the injection coincides with a normal appearance of cornified cells.

4. One or two injections did not induce oestrual changes in the vaginal mucosa of ovariectomized rats 600 days old. Nine injections caused such changes in ovariectomized rats 719 days of age. These changes appeared but once and lasted from two to four days.

5. Three to nine injections of the follicular hormone seemed to be favorable for both oestrual changes in the vaginal mucosa and mating in normal rats of various ages.

6. The results indicate that the hormone does not prolong the productive sexual life of the animal by inciting ovulation but that it may stimulate mating activities.

7. As a result of the hormone injections the majority of the animals showed a reduction in food consumption and spontaneous activity accompanied by an increase in body weight. This effect was of short duration, and a return to normal in a short time usually occurred.

8. All results indicate that the effect of the injected hormone is but transitory, lasting only a few days at the most.

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THE EFFECT OF STARVATION ON THE DAILY CONSUMPTION OF WATER BY THE DOG

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In a recent paper (Kleitman and Crisler) it was reported that starvation had a marked deleterious effect upon the salivary conditioned reflex in the dog. When the reflex was developed in a starving animal, the maximum quantity of saliva secreted conditionally was much smaller than what could be expected on the basis of the performance of other animals, and of the same animal on previous occasions, and in spite of the fact that the application of the unconditioned stimulus (in this case, daily injections of morphine) was kept up, the conditioned secretion gradually diminished in volume. The sudden enormous increase in the volume of the conditioned secretion upon realimentation suggested that the previous deterioration of the reflex was not due to partial atrophy of the salivary glands, but was in some way connected with the internal conditions that prevailed during the period of starvation. The animals were allowed to drink water *ad libitum*, and it was thought that perhaps they did not drink as much water when they were starved as when they were fed. That lack of water caused a pronounced decrease in the volume of saliva secreted conditionally was shown by some experiments reported in the above-mentioned paper. Thus, one of our dogs, M, was deprived of water as well as of food for two days (July 25–27), at a time when her salivary conditioned reflex was at its height. On July 25, the last normal day, she secreted 42.7 cc. in 30 minutes. On July 26 she secreted only 21.8 cc., and on July 27, 10.3 cc., having lost 0.9 kgm. in these two days. She was fed and watered on July 27, and on the following day she secreted 37.2 cc., and on July 29, 53.1 cc. in 30 minutes.

A careful search of the literature failed to reveal any data on the drinking habits of dogs, nor on the effect of starvation on water drinking in dogs. I decided then to determine by actual measurement the quantity of water consumed by dogs from day to day, both when fed and when starved. The periods of starvation were to be preceded and followed by normal control periods of sufficient length to bring out the difference, should there be any. Altogether six dogs were used in this investigation, and their daily consumption of water measured for a period of nearly two months. The

TABLE 1

Volumes of water drunk daily by six dogs, during alimentation, in starvation, and while receiving injections of pilocarpine

DATE	DAY OF WEEK	DOG M	DOG W	DOG T	DOG L	DOG H	DOG B
		cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
October 13	Wednesday	720	185	110	145	*10*	135
14	Thursday	420	230	140	*190*	465	225
15	Friday	185	475	100	*95*	165	275
16	Saturday	260	350	105	*200*	105	75
17	Sunday	*45*	*10*	*10*	*255*	*20*	*35*
18	Monday	280	350	235	*135*	440	445
19	Tuesday	135	310	235	*20*	90	205
20	Wednesday	305	480	210	*200*	495	250
21	Thursday	110	230	85	*30*	195	285
22	Friday	225	470	355	*230*	265	325
23	Saturday	370	185	400	*70*	295	415
24	Sunday	*15*	*10*	*10*	*150*	*90*	*90*
25	Monday	445	220	405	*370*	490	390
26	Tuesday	180	565	410	660	255	400
27	Wednesday	315	350	470	580	205	390
28	Thursday	*95*	505	*85*	365	*105*	*145*
29	Friday	*70*	140	*80*	240	*85*	*260*
30	Saturday	*90*	475	*105*	580	*190*	*235*
31	Sunday	*35*	385	*100*	305	*10*	*80*
November 1	Monday	*225*	375	*165*	580	*175*	*225*
2	Tuesday	*150*	405	*115*	405	*160*	*155*
3	Wednesday	*120*	305	*155*	300	*10*	*225*
4	Thursday	*235*	375	*215*	400	*160*	*210*
5	Friday	*175*	610	*25*	240	*135*	*275*
6	Saturday	*230*	320	*110*	255	*95*	*195*
7	Sunday	*25*	*70*	*125*	*10*	*5*	*170*
8	Monday	*200*	715	*165*	770+	*160*	*220*
9	Tuesday	*185*	545	*165*	335	*110*	*240*
10	Wednesday	*455**	520	*255*	595	*130**	*195*
11	Thursday	*190*	235	85	310	*165*	*215*
12	Friday	*180*	745	*155*	705	*165*	*250*
13	Saturday	*195*	500?	*95*	750	*165*	*555**
14	Sunday	*15*	*155*	*60*	*40*	*5*	*160*
15	Monday	530	370	570	265	305	435
16	Tuesday	370	300	475	320	125	430
17	Wednesday	95	320	350	455	210	320
18	Thursday	540*	650*	235	440	5	510*
19	Friday	210	105	610*	200*	240*	275
20	Saturday	295*	250*	250	290	135	470*
21	Sunday	*55*	*5*	*65**	*215**	*20**	*140*
22	Monday	500*	770+*	585	770+	290	440*
23	Tuesday	235	245	645*	610*	325*	245
24	Wednesday	265*	770+*	150	465	385	715*
25	Thursday	650	770+	770+*	770+*	730*	730

TABLE 1—*Concluded*

DATE	DAY OF WEEK	DOG M	DOG W	DOG T	DOG L	DOG H	DOG B
		cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
November 26	Friday	265*	690*	265	725	105	700*
27	Saturday	300	335	550*	520*	320*	265
28	Sunday	*145**	*170**	*65*	*175*	*185*	*420**
29	Monday	595	770+	725*	730*	455*	545
30	Tuesday	340*	420*	200	150	390	370*
December 1	Wednesday	365	440	300*	460*	175*	270
2	Thursday	335*	465*	480	285	490	365*
3	Friday	435	310	325*	770+*	270*	475
4	Saturday	225*	170*	500?	270	305	305*
5	Sunday	*60*	*95*	*200**	*245**	*10**	*140*
6	Monday	215*	295*	140	615	320	155*

Figures in italics indicate that the dog had not been fed on that day; figures marked with * indicate that the dog received an injection of pilocarpine.

dogs were generally not fed on Sunday, and besides this five of these dogs were deprived of food, one for 12 days, and four for 18 days continuously. All the animals had been in the laboratory for a long time and were accustomed to the environment. Their fare consisted of meat scraps from the University Commons, usually mixed and cooked with some bread. In each cage there were suspended from the wall two water cups of exactly the same size and shape, but one cup was covered with a wire-net lid. The water was changed once a day, at 9 a.m., and the same quantity of water was poured into each cup. By determining the volume of water that had disappeared from the cup with the wire-net cover, one could know just how much water had evaporated from the cup to which the dog had free access. This varied from 20 to 35 cc. in 24 hours and was always subtracted from the volume of water that the dog had apparently drunk.

The data obtained are presented in table 1. There was a great variation in the quantity of water consumed by any one dog from day to day. This may partly be due to the quantity of food received (which was by no means constant), and especially to the varying water content of the food. By drying some samples of food given to our dogs (at 110°C.) I found that the usual portion contained about 300 grams of solids and from 350 to 450 cc. of water. But the composition and water content of the food were not the sole factors concerned, as can be seen from the fluctuations in the volume of water drunk by the dogs during their fasting periods. The temperature of the air could not be a decisive factor, since the dogs were all in the same room, and yet one dog would drink more water than he did the previous day, and another less. These variations in the daily consumption of water by dog do not seem to be dependent upon any one known condition.

The average volume of water consumed by each of the dogs is not di-

rectly related to the size of the animal (table 2). In the cases of T and W, dogs of about the same size and weight, the average daily consumption of water was 298 cc. and 400 cc. respectively. Dog B is smaller than H, but he drank, on the average, 340 cc. of water per day, while H drank only 272 cc. Likewise L and M, of the same size, drank 437 cc. and 336 cc. per day respectively.

From table 1 it will be seen that the dogs generally drank surprisingly little on Sunday, the day on which they were not fed. What is very hard to account for is that even during continuous fasting the dogs often drank less water on Sunday than on either Saturday or Monday.

When the dogs were deprived of food for a number of days in succession, they drank more water daily than on foodless Sundays, but appreciably less than during the periods of alimentation. In table 2 are given the

TABLE 2

The average volumes of water drunk in 24 hours by six dogs on week-days, on Sundays during continuous starvation, and while receiving injections of pilocarpine (from data in table 1)

DOG	USUAL WEIGHT	WEEK-DAYS (FED)	SUNDAYS (NOT FED)	CONTINUOUS STARVATION	PILOCARPINE INJECTED (FED)
	kgm.	*cc.*	*cc.*	*cc.*	*cc.*
T	9–10	298 (25)	35 (3)	126 (18)	561 (7)
L	13–14	437 (31)	75 (3)	162 (12)	580 (7)
B	8–9	340 (23)	96 (4)	203 (17)	448 (9)
H	9–10	272 (24)	76 (4)	112 (17)	359 (7)
M	13–14	336 (23)	44 (4)	142 (17)	331 (9)
W	9–10	400 (39)	58 (6)		503 (9)

The figures in parentheses indicate the number of cases upon which the averages are based.

figures for the average consumption of water by five dogs during their fasts, and it will be noticed that the decrease in the volumes of water drunk in 24 hours amounted to 150 to 300 cc., or from 40 to 60 per cent of the normal. The gradual loss in body weight can account for but a portion of this decrease. Besides, the decrease in the volume of the water drunk is only a part of the decrease in the total water intake, since under conditions of alimentation the dogs get from 350 to 450 cc. of water with their food. Adding up these figures one finds that the total decrease in water consumption during fasting may equal to 500 to 700 cc., and that the 100 to 200 cc. of water drunk is really only from one-fifth to one-third total normal water intake. The decrease in total metabolism, entailing a diminution in the excretion of nitrogenous waste products and other crystalloids by the kidneys,with a consequent smaller volume of urine, would naturally call for a smaller water intake, but whether this can be held responsible for

the decrease of 65 to 80 per cent in the total water intake actually observed during starvation remains to be proven.

During the study of the salivary conditioned reflex the dogs were secreting large quantities of saliva daily. It was interesting to see if secretion of such quantities of saliva would affect the drinking habits of the dogs. Accordingly the dogs were given subcutaneous injections of pilocarpine (0.5 mgm. per kilo) every other day, and the volumes of water drunk on such days compared with the figures for the normal days. It should be mentioned that the usual volume of saliva secreted as a result of the injection of pilocarpine varied from 100 to 200 cc. It was found that on the days they received pilocarpine all the dogs but one drank, on the average, much more water than usual (table 2). This increase in water consumption is probably due to the loss of water through secretion of saliva, but it may also be due to some other effects of pilocarpine.

The purpose of this investigation was to determine whether dogs drink less water when they are starved than when they are fed. The results show that that is the case, and that the decreased water intake may be an important factor favoring the gradual deterioration of the salivary conditioned reflex observed under these circumstances. In another communication I expect to be able to point out other possible factors that may contribute to the practical abolition of the salivary conditioned reflex in starvation.

SUMMARY

1. The water intake of dogs varies from day to day and is not directly related to body weight, diet or temperature of the environment.

2. In starvation dogs drink about one-half the quantity of water they drink during alimentation, but their total water intake is only about one-fifth to one-third of the normal.

3. Subcutaneous injection of 0.5 mgm. of pilocarpine per kilo usually results in an increase in the volume of water taken on that day.

4. The marked decrease in the water intake in starvation is probably one of the causes of the gradual deterioration of the salivary conditioned reflex observed in that condition.

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THE EFFECT OF SOME SECRETAGOGUES ON THE CHEMICAL COMPOSITION OF THE PANCREATIC JUICE

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Although many workers have studied the effects of various secretagogues on the activity of the pancreas, but few have noted the effects of the former on the chemistry of the resulting secretion. In this paper we shall present the results of a study of the chemical character of the pancreatic juice subsequent to the administration of three different types of secretagogues: i.e., secretins, with and without the vasodilatin fraction; pure vasodilatins, i.e., $NaNO_2$ and peptones, and parasympathetic stimulants, i.e., pilocarpine.

Frequently, earlier observers have published the results of one or more secretagogues as obtained from one animal. However, since we have observed a relatively large variation in the secretory response of different dogs to similar dosages of the same secretagogue, a sufficiently large number of animals has been included in each series to obviate such a possible error and at least three different secretagogues have been tested on each of the 52 dogs used.

LITERATURE. Dolinski (1894) showed that a 0.4 per cent solution of hydrochloric acid placed in the gut would cause an increased secretion of the pancreas. Bayliss and Starling (1902) showed than an acid extract of the duodenal mucosa would if injected intravenously cause pancreatic secretion. Popielski (1906) in a number of papers questioned the physiologic significance of Bayliss and Starling's discovery on the ground that acid extracts of nearly any tissue would under similar conditions cause the pancreas to secrete. He noted the vasodilatation caused by all of the extracts and believed the vasodilatation in the pancreas was the cause of its secretion. Koch, Luckhardt and Keeton (1920) showed the similarity of gastric and intestinal extracts on gastric secretion, being unable to confirm the work of Edkins. E. Parsons (1925) was unable to confirm the theory that secretin activity was due to the histamine content of secretin preparations. She also stated, "The secretin preparations do not appear to represent a true physiological process."

Bayliss and Starling (1904) compared some of the properties of pancreatic juice after the injection of secretin and pilocarpine, and observed

that the pilocarpine juice was richer in protein, salts and titratable base. Dubois and Polonovski (1925) observed that pilocarpine and eserine juices have a lower pH than secretin juice. Marie Skarzynska (1924) concluded that secretin is three times as efficient a pancreatic secretagogue as histamine or gastrin, and that the effect is not due to vasodilatation. Cohnheim found normal pancreatic juice of dogs to contain 0.31 per cent of sodium carbonate and 0.66 per cent of sodium bicarbonate. Bierry (1907) found that secretin juice from a dog with a temporary fistula contained sodium carbonate equivalent to an N/10 solution.

De Zilwa (1904) found that secretin juice contained from 1.6 to 2.2 per cent of solids while pilocarpine juice contained 6.4 per cent of solids. He found the titratable base to be from 9 ml. to 14 ml. of N/10 base per 100 ml. of juice, the secretin juice being more alkaline than the pilocarpine juice. E. Terrioine (1913) experimenting on dogs found that secretin juice was richer in enzymes than juice collected after a meal. However, he concludes that secretin juice is very similar to the normal juice. McClure (1924) found the fasting human duodenum to contain a juice which varied very widely in both enzymes and solids.

Farrell and Ivy (1926) found that various foods caused the pancreatic transplant to secrete varying quantities of juice but the quality remained fairly constant.

Weaver, Luckhardt and Koch (1926) have prepared and studied the action of a vasodilatin-free secretin. Mellanby and Huggett (1926) express the hypothesis that the pancreas is under vagus control and that the vagal juice contains more enzymes than secretin juice. Secretin causes a copious flow of sodium bicarbonate which carries the enzymes with it. Mellanby (1926) has prepared a crystalline compound from intestinal mucosa which he believes is pure secretin.

Experimental. Large adult dogs were used in all of these experiments, the weights being between 14 and 20 kgm., since young animals were found to be unsuitable. The animals were prepared in the following manner: They were first anesthetized by means of ether, then 225 mgm. of sodium-barbital per kilogram were given by vein. The abdomen was opened and after cannulating the duct of Santorini, the pylorus was tied off and the incision closed with hemostats. A cannula was inserted into the femoral vein through which all injections were made. The bile-free pancreatic juice was collected in small g.s. bottles which were easily kept cold until ready for analysis. All analyses were made within three hours after collection of the juice. Care was taken that recovery from the effects of one secretagogue, as evidenced by the blood pressure level and pancreatic secretory rate, should be complete before a second secretagogue was administered. Also the pancreatic duct cannula was emptied at the end of each

collection, so that each sample of juice represented the secretion from only one secretagogue.

Bayliss and Starling secretin was used as the standard in the comparison of our results. This secretin preparation was injected alternately with the other preparations in order that we might have a control for each sample of juice.

Four types of secretagogues were used:

I. Secretin preparations:

a. Bayliss and Starling (BS), dose 5 ml.

b. New secretin[1] (new), dose 5 ml.

c. Vasodilatin-free secretin[2] (W), dose 5 ml.

II. Vasodilatins:

a. Sodium nitrite ($NaNO_2$), dose 20 mgm.

b. Witte peptone 5 per cent (Peptone,) dose 25 ml.

III. Parasympathetic nerve ending stimulants.

a. Pilocarpine HCl (Pilo), dose 20 mgm.

IV. 0.2 per cent HCl in the gut (HCl), dose 100 ml.

METHODS OF ANALYSIS. *1. Specific gravity.* The specific gravity of the juice was determined by means of Hammerschlag's method, i.e., suspending a drop of the secretion in a mixture of benzene and chloroform, adding either benzene or chloroform to the mixture until the drop remained stationary. The specific gravity of the mixture was then determined.

2. Hydrogen ion concentration. The pH was determined by means of the hydrogen electrode.

3. Titratable base. Two milliliters of the juice were diluted to 10 ml. with water and titrated to the end point of methyl orange with N/10 HCl.

4. Lipase. The ethyl butyrate method for determining lipase proved to be quite satisfactory (Farrell and Ivy, 1926). We used 5 ml. of absolute ethyl butyrate, 9 ml. of water and 1 ml. of pancreatic juice. Four drops of toluol were added to prevent bacterial action. The containers were rotated in a rotary shaker at 40°C. The liberated acid was titrated with N/10 NaOH using phenolphthalein as the indicator. The proper controls were carried out.

5. Trypsin. The method of Koch and Helmer (1926) was used for the determination of trypsin. Eighty milliliters of a $7\frac{1}{2}$ per cent solution of casein (best grade Hammarsten) dissolved in 0.4 per cent sodium carbonate was placed in a 150 ml. Erlenmeyer flask. To this was added an equivalent of 2 ml. of the activated[3] pancreatic juice and the volume made to 100

[1] This preparation was prepared by the method of Luckhardt, Barlow and Weaver (1926).

[2] Prepared by the method of Weaver, Luckhardt and Koch (1926).

[3] To activate the pancreatic juice 4 ml. of the juice were neutralized and made up to 8 ml. with water. To this add 2 ml. of an enterokinase solution (2 per cent). After 15 minutes add 5 ml. to the casein mixture (an equivalent of 2 ml. of juice).

ml. with 0.4 per cent sodium carbonate. Twenty-five milliliters of the mixture were immediately removed and acidulated with 5 ml. of N/1 acetic acid to precipitate the casein. After shaking with 2 grams of talc this fraction was filtered and the refractive index determined on the filtrate at 25°C. The remainder of the substrate-enzyme mixture was rotated in a rotary shaker at 40°C. for exactly four hours. At this time a second 25 ml. sample was removed and treated as before. The difference in the two refractive index readings was converted into milligrams of "pancreatin" by means of a table prepared by Koch and Helmer.

Results. Tables 1 to 6 show the average result on the basis of the data obtained from all of the dogs, comparing each secretagogue, per period, to the nearest control. A "period" in these tables represents two separate analyses, namely, a BS secretin juice and the juice which immediately follows it.

Table 7 shows the average result for each secretagogue on all dogs, compared with the average of the control (BS secretin standard) in individual experiments.

Table 8 shows the composite average results of each secretagogue employed. In other words, this table contains the average results obtained from all of the dogs used in these experiments.

Table 9 shows the summary of our findings. The secretagogue ratio, trypsin ratio, and lipase ratio were determined by dividing the units secreted after one dose of a given secretagogue by the units secreted after one dose of BS secretin. The figures used in determining these ratios were the composite averages from all of our experiments. Comparisons such as we have made in this table show only the relationship of the effects of unit doses of the preparations and can not be taken as absolute comparisons of efficiency since we do not have a basis for making such comparisons.

Discussion. Our finding that pilocarpine juice contains more solids than secretin juice is in agreement witb Bayliss and Starling, and De Zilwa. We could not confirm Bayliss and Starling's finding that pilocarpine juice is more alkaline than secretin juice. We found secretin juice to contain more titratable alkali than pilocarpine juice, which is in agreement with De Zilwa. Our finding that pilocarpine juice has a lower pH than secretin juice confirms the work of Polonovski. We have confirmed Terrioines' finding that secretin juice contains a higher concentration of enzymes than juice secreted after a meal (HCl in gut).

There appears to be a rather characteristic type of juice from each type of secretagogue used. The three secretins (BS, New and W) cause a secretion of medium specific gravity and high in alkalinity, while the vasodilatins (Witte peptone and $NaNO_2$) cause secretions of high specific gravity and somewhat lower alkali content. Pilocarpine causes a secretion of very high specific gravity, due to the high protein content, and with a lower alkali content than the secretin juice.

TABLE 1

Comparing the pancreatic juice obtained from HCl acting on the duodenal mucosa with the pancreatic juice obtained from intravenous injection of BS secretin

SECRETAGOGUE	NUMBER OF DOGS	PERIOD	PH	BASE ML. N/10 NAOH PER 100 ML.	MILLIGRAM TRYPSIN PER 100 ML.	LIPASE ML. N/10 NAOH	SPECIFIC GRAVITY
BS	6	1	8.41	135	237	2.0	1.018½
HCl	5	1	8.1	134	127	1.8	1.019½
BS	5	2	8.12	135	250	2.0	1.017½
HCl	5	2	8.15	135	128	1.9	1.021

TABLE 2

Comparing the pancreatic juice following stimulation with pilocarpine and with BS secretin

SECRETAGOGUE	NUMBER OF DOGS	PERIOD	PH	BASE ML. N/10 NAOH PER 100 ML.	MILLIGRAM TRYPSIN PER 100 ML.	LIPASE ML. N/10 NAOH	SPECIFIC GRAVITY
BS	5	1	8.4	125	147	1.8	1.022
Pilo	5	1	8.2	115	187	1.8	1.026
BS	5	2	8.24	140	170	2.0	1.020
Pilo	5	2	8.1	115	197	1.6	1.025
BS	3	3	8.2	138	186	1.9	1.020

TABLE 3

The character of the pancreatic juice under Witte peptone and BS secretin stimulation

SECRETAGOGUE	NUMBER OF DOGS	PERIOD	BASE ML. N/10 NAOH PER 100 ML.	MILLIGRAM TRYPSIN PER 100 ML.	LIPASE ML. N/10 NAOH	SPECIFIC GRAVITY
BS	6	1	134	229	1.8	1.020
Peptone	6	1	135	442	1.6	1.024
BS	3	2	115	285	1.8	1.019
Peptone	3	2	87	340	1.6+	1.025
BS	3	3	82	142	1.85	1.020
Peptone	3	3	115	332		

TABLE 4

Comparing the pancreatic secretion under "New" secretin and BS secretin stimulation

SECRETAGOGUE	NUMBER OF DOGS	PERIOD	BASE ML. N/10 NAOH PER 100 ML.	MILLIGRAM TRYPSIN PER 100 ML.	LIPASE ML. N/10 NAOH	SPECIFIC GRAVITY
BS	7	1	111	240	2.1	1.014
New	7	1	120	203	2.3	1.016
BS	7	2	115	205	1.25	1.017½
New	6	2	117	200	1.7	1.018
BS	3	3	118	211		
New	2	3	114	170		

TABLE 5

Comparing the pancreatic secretion under sodium nitrite and BS secretin stimulation

SECRETAGOGUE	NUMBER OF DOGS	PERIOD	BASE ML. N/10 NAOH PER 100 ML.	MILLIGRAM TRYPSIN PER 100 ML.	LIPASE ML. N/10 NAOH	SPECIFIC GRAVITY
BS	5	1	135	165	2.25	1.019
$NaNO_2$	5	1	118	193	0.95	1.020
BS	5	2	138	170	2.20	1.020
$NaNO_2$	5	2	115	187	1.35	1.017
BS	4	3	132	137	1.70	1.019
$NaNO_2$	5	3	110	152	1.75	1.023

TABLE 6

Comparing the pancreatic secretion under "W" secretin and BS secretin stimulation

SECRETAGOGUE	NUMBER OF DOGS	PERIOD	BASE ML. N/10 NAOH PER 100 ML.	TRYPSIN MILLIGRAM PER 100 ML.	LIPASE ML. N/10 NAOH	SPECIFIC GRAVITY
BS	9	1	125	230	2.1	1.014
W	9	1	120	139	3.0	1.018
BS	7	2	130	174	1.25	1.017
W	7	2	120	138	2.2	1.018

TABLE 7

Comparing the average for each secretagogue with the average of the BS secretin control

SECRETAGOGUE	PH	BASE ML. N/10 NAOH PER 100 ML.	TRYPSIN MILLIGRAM PER 100 ML.	LIPASE ML. N/10 NAOH	SPECIFIC GRAVITY
BS		114	271	2.1	1.016½
New		115	208	2.7	1.015
BS		123	196	2.1	1.016½
W		120	133	2.6	1.017½
BS	8.3	131.5	155	1.8	1.022
Pilo	8.12	115	193	1.8	1.025
BS	8.3	92½	255	1.8	1.020
Peptone	8.08	115	408	1.6	1.024
BS		135	147	1.9	1.020
NaNO		118	183	1.5	1.020
BS	8.31	156	109	1.85	1.019
HCl	8.15	135	127	1.9	1.019½

Probably the differences found in the three secretin juices are due to the differences in vasodilatin content of the secretin preparations.

The lipase seems to be related to the water fraction of the juice, i.e., those juices collected after stimulation by secretagogues which cause a more watery secretion of low specific gravity contain higher concentrations of lipase. Those secretagogues which cause the secretion of juice rich in proteins and of high specific gravity produce juices which are relatively high in trypsin (ogen) content. Especially is this true in the case of Witte peptone, the effects of which are probably due to the toxic cleavage products of the protein. Luckhardt (1926, personal communication) found that highly purified peptone was not a satisfactory secretagogue and he attributes this difference to the difference in the methods of preparation of the two peptones, i.e., the Witte product being made from scraps, etc., while the C.P. peptone is made from clean fresh protein.

TABLE 8

Composite table showing the average result for each secretagogue

SECRETAGOGUE	NUMBER OF DOGS	EXPERIMENTS AVERAGED	PH	BASE ML. N/10 NAOH PER 100 ML.	TRYPSIN MILLIGRAM PER 100 ML.	LIPASE ML N/10 NAOH	SPECIFIC GRAVITY
BS	42	82	8.3	130	195	1.9	1.020
New	14	24	8.13	115	212.5	2.7	1.015
W	13	32	7.85	120	143	2.6	1.017½
Pilo	6	16	8.12	113	192½	1.8	1.026
Peptone	6	12	8.08	115	408	1.6	1.024
$NaNO_2$	5	18		115	185	1.5	1.020
HCl	6	11	8.11	135	126	1.9	1.019

TABLE 9

SECRETAGOGUE	SECRETAGOGUE RATIO	BASE, AVERAGE ALL DOGS	PH, AVERAGE ALL DOGS	SPECIFIC GRAVITY, AVERAGE ALL DOGS	TRYPSIN RATIO	LIPASE RATIO
BS	1	130	8.3	1.020	1	1
Pilo	1.57	113	8.12	1.026	1.24	1
Peptone	0.725	115	8.08	1.024	1.5	0.92
$NaNO_2$	1.15	115		1.020	1.24	0.77
New	0.75	115	8.13	1.015	0.76	1.2
W	0.2	120	7.85	1.017½	0.70	1.2
HCl	1	135	8.11	1.019	1.1	1.03

SUMMARY

Data are given showing the effect of several secretagogues on the chemistry of the pancreatic juice.

The authors wish to express their gratitude to Dr. A. B. Luckhardt for suggesting this problem and for his advice and assistance during the work, and to Dr. F. C. Koch for his advice and assistance in the method for determining trypsin.

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STUDIES OF THE THYROID APPARATUS

XLVII. The Cyclic Character of the Response to Parathyroid Deficiency

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In 1923 attention was directed to the fact that growth of the albino rat in body weight in conditions of parathyroid deficiency exhibits alternating periods of decrease and increase in rate (1). This observation was made on male and female animals parathyroidectomized at 100 days of age. In 1926 a survey of the individual growth curves of the rats of the several other groups parathyroidectomized at 23, 30, 50, 65 and 75 days of age showed that the cyclic character of growth is a phenomenon of general occurrence under these conditions (2). It is as frequently expressed by alternating periods of loss and gain in weight, as by decreases and increases in growth capacity. The actual gains in weight are, however, usually greater than the losses, so that the animal at the end of the period of observation is heavier than at the beginning. In 1924 I recorded the fact that the occurrence of broken incisor teeth in parathyroidectomized rats is also cyclic (3). That is to say, a tooth which becomes broken may be repaired by continued growth, only to be broken again, and again repaired during the course of the life of the animal after the initiation of the glandular deficiency.

Recently Sloan (4) has presented data which show "that the phenomenon of parathyroid tetany is cyclic in character," thus confirming my earlier generalization.

These facts have considerable bearing on any explanation of what is happening in an animal deprived of its parathyroids. Therefore it seems worth while to put definitely on record a few specific examples drawn from my data. It is not necessary to give all the protocols. Two or three will suffice to demonstrate the basis of the principle it is desired to establish.

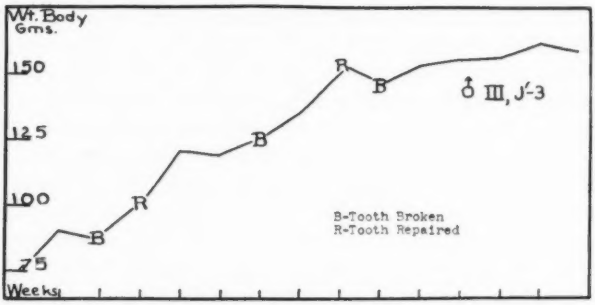
Rat III, F'-3, male, 30 days old, was parathyroidectomized on May 4, 1923. An incisor was first found broken on June 23: repaired on June 30: broken again on July 14: repaired again on July 21, and broken again on July 28.

Rat III, F'-4, male, 30 days old, was parathyroidectomized on May 4, 1923. An incisor was first found broken on June 16: repaired on July 14: broken again on July 28: repaired again on August 11, and broken again on August 18.

Rat VI, J'-4, male, 65 days old, was parathyroidectomized on April 21, 1923. On June 16 an incisor was first found broken: repaired on June 26: and broken again on July 2.

A tracing back of this succession of breakages and repairs brings out the fact that it follows closely, in practically every case, the alternating periods of decrease and increase in rate of growth in body weight. An example is given in the chart of rat III, J'-3, a male, parathyroidectomized at 30 days of age.

It is seen here that the weakness of tooth, caused either by deficient calcification or de-calcification, which results in breakage after a preliminary condition of opacity, follows a period of weakness in body growth, when growth capacity is decreased below that of the preceding period or retrogressive with actual loss of weight: and that tooth repair, or recovery from weakness, takes place shortly after body growth has become stronger



The curve shows the correlation between the periods of growth depression and tooth breakage, and the periods of growth acceleration and tooth repair.

or increased in rate over the preceding period, and the capacity become positive.

This trailing parallelism between the viscissitudes of tooth and body weight growth and recovery is sufficiently consistent in its appearance and uniform in nature to justify the conclusion of an associative relationship and a common causative factor.

That the breaking and repair should come after the corresponding expression of alteration in body growth is but natural since the tooth material, like that of the bones (5), is relatively stable and presumably more resistant to the growth inhibiting effects of parathyroid deficiency than the body as a whole, and since tooth growth rate is slower than body growth rate even under normal conditions (6) (2).

The concurrence of these phenomena establishes the cyclic character of the reaction of the organism to parathyroid deficiency as something which

can not be pushed aside as negligible in any interpretation of the happenings which follow parathyroid removal.

The type of change noted above is suggestive of a flowing and ebbing of a toxic condition. The sharp upturn in the growth rate after the period of retardation or regression is indicative of a release from a previous inhibition.

There is as yet to be recorded an hypothesis of the mechanism productive of the several phenomena incident to parathyroid deficiency, which is anything but fragmentary and grossly incomplete Each interpreter has tended to confine his deciphering to the hieroglyphics immediately within his ken, and has disregarded those which other workers are attempting to render intelligible. But the ultimate solution of the code obviously rests upon a correlation of the several groups of facts. By no other means can an intelligent idea of the meaning of the picture be obtained.

Although the evidence available from which an interpretation of the cyclical character of the growth, tooth defects, and tetany of parathyroid deficiency can be formulated is far from sufficient or satisfactory, the following suggestion is given in an attempt to not only explain this phase of the subject, but also to afford a basis for the explanation of the several hitherto and apparently unrelated reactions.

The evidence is indisputable that the calcium balance within the organism is related to parathyroid activity (7). Equally potent evidence indicates that a function of the parathyroids is concerned in the prevention of the accumulation of, or in the facilitation of the disposal of, toxic products of exogenous (8) (9) or endogenous (10) origin.

The fact that blood calcium is immediately raised when parathyroid extract is administered (7) (11) indicates that the low blood calcium of parathyroid deficiency is hardly to be considered as a secondary reaction to the toxemia. The fact that tetany does not necessarily occur in conditions of parathyroid deficiency even when the blood calcium level is low, as shown by Sloan (4) and Jones (12), indicates that the disturbance of the calcium balance is not the sole determinant of the tetany, though it indeed may be a contributory factor. It is difficult to see how the disturbance of calcium equilibrium can be assumed to be the primary or initiating agency in the growth inhibition.

The present confusion may be due to the possibility that there are two separate functions mediated by the parathyroids. One concerned in the regulation of the calcium balance, the other in the prevention of the accumulation, or the facilitation of the disposal, of toxic products resulting from intermediary or preparatory metabolism.

Granting the assumption for purposes of exposition if for nothing else, the significance of the course of events may be pictured as follows.

Parathyroid removal lowers the ability of the organism to dispose of toxic tetany-producing compounds, or removes a check on their produc-

tion. These gradually accumulating produce an increasing severity of tetany. They, also, being neuro-toxins, so act on the visceral nervous system that digestive processes are inhibited (13). The restriction of food absorption and the amount of metabolites which might otherwise be used for growth either directly or substitutively, but which are burned up by the maintenance demands of the tetany, combined with the possible influence of the toxic products themselves on the body cells, results in a condition of virtual undernutrition which brings about a retardation of growth.

Through the loss of the calcium-balance regulating function of the parathyroids blood calcium is lowered (14). Contributory to this is the probable disturbance of calcium absorption (15) or excretion (16) through the intestinal wall, due to the action of the toxic compounds thereon as well as to their action on the viscero-neural system.

The accumulation of the toxic products finally precipitates an attack of acute tetany, which is probably bolstered by the calcium deficiency. The metabolic intensity of the tetanic seizure burns up or in some other way facilitates the disposal of the toxic compounds, and releases the cell inhibition to growth. The brake on the viscero-neural system is released, digestion approaches its normal intensity, more food is absorbed than is needed for maintenance and body growth proceeds at a more rapid rate.

During the period of growth retardation the low blood-calcium combined with the state of low nutritional level, which of itself is known to retard osseous development, fails to provide adequate calcium for the growing incisors. They, therefore, become deficiently calcified (17) and being fragile break off. This reaction is delayed for the reasons given in an earlier paragraph.

With the temporary release from the toxemia, growth of the broken, deficiently calcified teeth continues with an improvement in the degree of calcification. This is evident from the fact that the new growth produces unbroken incisors of approximately normal size and durability. It occurs in spite of the actual continuance of the parathyroid deficiency. This is noteworthy and implies a tendency of readjustment toward the normal calcium balance in so far as tooth growth is concerned. The improvement in dental calcification may be due either to a raising of the blood-calcium level, which is improbable (4), or to the release from the toxemia of the intestinal excretory or absorption processes, combined with a selective utilization by the teeth of the additional calcium thus made available. This might well occur and the figures found for the blood-calcium still be low. An added factor of importance is obviously the improved nutritional level.

Such is the cycle which may be repeated as long as the animal survives.

That parathyroid deficiency is present even during the period of renewed body growth activity and tooth repair, and that these recoveries

are not due to a compensatory functional activity of hypothetical accessory parathyroid tissue, can be inferred from the fact that the recovery is not maintained, but body growth again becomes retarded and the teeth again become broken, to again recover and again "zu grunde gehen." If the recovery from the first acute deficiency were due to a compensatory functional activity of the hypothetical accessory parathyroid tissue, this should be maintained and not be intermittent.

This seems as good a place as any to set on record the hints of the presence of a seasonal influence on the occurrence of teeth defects in conditions of parathyroid deficiency. The analysis of the data was made from the point of view of the relation between the month of operation and the time elapsing between parathyroid removal and the appearance of broken teeth; from the point of view of the relation between the percentage incidence of teeth defects and the month of operation; and from the point of view of the monthly percentage incidence of broken teeth. Although the results of the analyses considered individually reveal data which are only suggestive, when combined they give evidence for the opinion that seasonal influence is a factor in the determination of the onset of the dental defects.

Thus broken teeth appear after an interval of 57 days in the average when the parathyroids are removed during the months of March, April or May. From then on the interval between operation and appearance of dental defects tends to lengthen until it reaches the value of 73 days on the average in the rats parathyroidectomized during January and February. The change from February to March is thus seen to be abrupt. Although the increase from May to January is not steady it is traceable. Assuming for the moment that it is real, it indicates that some change has been produced in the rats so that beginning with the month of March and continuing through April and May, the capacity for teeth calcification in conditions of parathyroid deficiency is lowered below the level existing immediately before so that the onset of broken incisors is hastened.

Consistent with this is the fact that whereas the percentage incidence of broken teeth in the rats operated in January, February, March and April is about 59, 42, 67 and 61 per cent respectively, the values for May and June jump up to 100 and 75 per cent respectively, and hereafter drop again to the lower figures of 40, 57, 63, 59, 71, and 50 for the rats parathyroidectomized in July, August, September, October, November and December. The high percentage incidence of broken teeth in rats operated in May and June as compared with the preceding and succeeding months might well be a consequence of conditions lowering the resistance during the months immediately preceding the initiation of the glandular deficiency.

Finally the data indicate that the highest percentage incidence of broken teeth occurs in the months of July and August. These are times of high temperature and high humidity in Philadelphia. To this may possibly be attributed the greater incidence of these dental defects. Although the

number of animals used in these analyses are inadequate for differential statistical analysis, it is not improbable that the tendencies noted are real indications of seasonal differences in susceptibility to the lack of the calcium balance regulating function of the parathyroids.

The suggestion that seasonal conditions during the months of March, April and May so affect the organism that parathyroid deficiency is more intense in its effect on the teeth-calcifying processes, is consistent with, though not necessarily related to, the prevalent opinion that an exacerbation of the condition known as idiopathic tetany is also observed in these early spring months. The temporal association of the two diverse phenomena may or may not indicate that the latter is to be in part attributed to a parathyroid deficiency. The relation, however, is suggestive.

CONCLUSION

Body growth and tooth calcification in the parathyroidectomized albino rat run a cyclical course of retardation and acceleration, or breakage and repair in which the changes in tooth condition follow those of the body growth rate in training parallelism. An interpretation is suggested of the sequence of events and other phenomena exhibited in conditions of parathyroid deficiency based on the idea that the parathyroid glands subserve two functions, that of participation in the regulation of the calcium balance, and that of the prevention of the accumulation of toxic tetany producing compounds within the organism.

A possible seasonal difference in sensitivity to parathyroid deficiency is indicated.

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MUSCULAR RHYTHMS AND ACTION-CURRENTS

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Although the periodic nature of neural discharge has been recognized for some time, only recently has the tetanic nature of all voluntary movement been appreciated.

Piper (1912), Forbes (1924), Cobb (1918), Adrian (1925), Athanasiu (1923) and others have shown that the activity of muscles and nerves gives rise to an action-current which has for its chief characteristic the presentation of a number, more or less large, of oscillations per second. The number varies between 50 and 500.

Hill (1921), by aid of the hot wire sphygmograph, was able to record in the string galvanometer the vibrations of the muscles of the human body and found them to appear as rapidly as 50 per second. His records bear a strong resemblance to those of Piper for the electrical change and he concludes that they are due to the same cause, that is, to a tetanus provided by the central nervous system.

Athanasiu found in action currents from the forearms during voluntary flexion of the digits a periodic grouping of the oscillations, the groups occurring about 10 times per second. He believed these periods were due to discharges from the Betz cells of the pre-central gyrus.

The present study attempted to determine the similarity between action current records and records of muscular rhythms. In order to make the latter records valuable for comparison with the detailed pictures one is able to obtain of action currents, a highly refined technique for amplification of extremely minute movements must be employed. That is, few if any displacements of the part studied should escape being recorded. The writers are not aware of any workers having recorded oscillations of a muscle or displacements of a part such as a finger, at a rate exceeding 50 per second. If this rate is the maximum for muscular rhythms then these rhythms do not bear a very close correspondence to electrical changes in contracting muscle. It was thought probable however that 50 per second did not represent the highest rate of muscular oscillations but only indicated the inadequacy of the recording methods.

The apparatus adopted for this study consisted in the main of an ampli-

fier, two phonelescopes[1] and a carbon button. A three stage impedance coupled amplifier was used. It consisted of one stage of high and two of low or power amplification. Shielding was found necessary in order to eliminate magnetic coupling within the amplifier itself, and to eliminate stray magnetic and electric fields. An input transformer was used in all instances to obtain greater stability and sensitivity. The electrodes for the forearm consisted of two German silver plates about 27 mm. in diameter, covered with canton flannel, which was soaked in a concentrated salt solution. For the tongue the bare ends of the input wires served as the electrodes. In picking up the muscular rhythms a carbon button in circuit with a broadcast microphone transformer was connected to the input of the amplifier. The carbon button was mounted about 1 meter from the floor against a solid concrete wall, 13 inches in thickness. A phonelescope with an electro-magnetic type of telephone receiver screwed in its back served as the recording instrument for both the action currents and the muscular rhythms. It was placed directly in the plate circuit of the last tube. Time was recorded by running a sixty cycle current through a stepdown transformer (110/24) and 2 m.f. condenser to a phonelescope with an electro-magnetic type of telephone receiver. Eastman standard size, super-speed moving picture film was used.

Several control experiments were made to test the stability of the apparatus and its freedom from inherent periodicities and extraneous currents and jars. First the input of the amplifier was short circuited.

[1] "Phonelescope" is the trade name of a standard instrument designed and sold by H. G. Dorsey, 1 Essex Ave., Gloucester, Mass. Briefly, it is an optical lever. A very small mirror mounted on jewel bearings is activated by a sensitive membrane. A beam of light focused upon the movable mirror is reflected to the photographic film.

Fig. 1. The amplifier input short-circuited. The output controlled the first line above the time line. The partials in the time line were due to the fifth harmonic of the sixty cycle alternating current. Some of the records may show the presence of the seventh harmonic. The second straight line above the time line is a "check line" to furnish a constant check on the rigidity of the recording apparatus.

Fig. 2. Before and after contraction began. The "check line" is at the lower margin of the film.

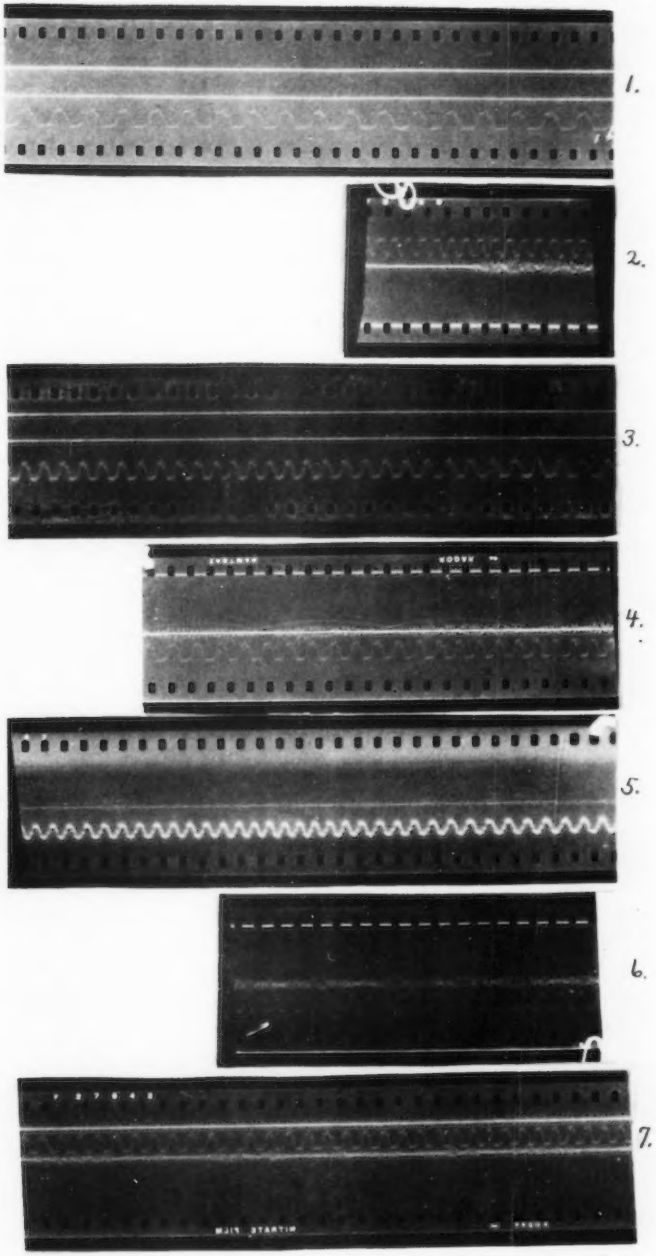
Fig. 3. Constant mechanical pressure on the carbon button indicated by first line above time line. Second line is "check line."

Fig. 4. Action currents from the dorsal surface of the tongue during voluntary elevation of the organ. A zero axis and a boundary line have been drawn in the record. The "check line" appears at the upper margin of the film.

Fig. 5. Muscular rhythms of the tongue.

Fig. 6. Action currents from the right forearm during voluntary flexion of the digits.

Fig. 7. Muscular rhythms from pressing fore-finger of right hand against carbon button.



Figs. 1–7

This would furnish a check on any periodicities characteristic of the amplifier itself. About thirty feet of film were taken under this condition, a sample of which is shown in figure 1. Here it is seen that there is not the slightest evidence of a disturbance in the apparatus. Second, the electrodes were placed on the relaxed tongue or arm and allowed to remain there for a considerable length of time before contraction was begun. Figure 2 is a sample of film showing the absolute quietness of the apparatus before contraction begins. Third a picture was taken with a constant pressure on the carbon button. This is shown in figure 3, where it is seen that no oscillations of any sort are recorded. It would appear then that the periodicities and oscillations to be reported represent true electrical changes in the contracting muscle and genuine muscular rhythms.

Figure 4 presents a record of action currents taken from the dorsal surface of the tongue during voluntary elevation of the organ. This and all subsequent records show a modulated wave, that is, a wave having the amplitude of its oscillations varied periodically. It is fairly symmetrical with respect to the zero axis; that is, the part of the modulated wave below the zero axis is a reflection of the part of the wave above the zero axis. This modulated wave is made up of an audible or principal frequency and an inaudible frequency. The inaudible frequency (10 to 12 per second) is composed of the periodic variations in intensity of the audible frequency (300 to 600 per second) and is termed the "envelope." This envelope shows the nature of the electrical change in the audible frequency.

For clarity's sake a penciled boundary and an inked axis have been drawn in figure 4.

Figure 5 is a record produced by protruding the tongue and gently pressing it against the carbon button. The striking similarity between this picture and that of action currents from the tongue is at once evident. The envelopes occur at the rate of about 10 per second while the rapid oscillations within each envelope vary between 300 and 600 per second.

Figure 6 shows action currents taken from the right forearm during voluntary flexion of the digits. The envelopes although not quite as distinct as those for the tongue, appear at the rate of about 10 or 12 per second while the oscillations in each envelope occur within the usual range of 300 to 600 per second.

Figure 7 is a record of muscular rhythms obtained by gently pressing the fore-finger of the right hand against the carbon button. The envelopes at the rate of 10 or 12 per second are clearly shown and the high frequency oscillations making up each envelope occur at a rate varying from 300 to 600 per second.

All of these records were taken from one normal individual. Records taken from a considerable number of other subjects, however, presented the same characteristics.

From the foregoing pictures it seems reasonably safe to suppose that there are two or more discharging centres, one furnishing the audio or principal frequency and the other the modulating inaudible frequency which causes the envelopes.

The striking similarity existing between the action current records and the pictures of muscular rhythms would point to the conclusion that a muscle responds almost perfectly to the electrical changes giving rise to the action currents.

More careful analysis of already existing records and further intensive work along these lines may demonstrate other audio-frequencies and modulated waves.

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THE RELATION OF THE T WAVE TO THE ASYNCHRONISM BETWEEN THE ENDS OF RIGHT AND LEFT VENTRICULAR EJECTION

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Although it is well substantiated that the T wave occurs near the end of systole, no consistent time relation between the two could be established by Garten and Sulze (1916), Wiggers and Dean (1917), Bartos and Burstein (1924) and Sands (1923). Inasmuch as one of us (Katz, 1925) has shown that the end of systole is not synchronous in both ventricles, we attempted to determine if a more consistent time relation would be found on comparing the T wave with the end of both right and left ventricular systole.

Furthermore, we tested out the hypothesis, suggested by Burdon-Sanderson and Page (1880), and later by Bayliss and Starling (1892), Einthoven (1913) and others, that the usual upright character of the T wave is due to a continuation of negativity and contraction of the right ventricle (base of heart) after it has disappeared from the left ventricle (apex). One of us (Katz, 1925) actually showed that the asynchronous cessation of activity of the two ventricles, postulated by this hypothesis, did occur, the right ventricle as a rule continuing to eject blood after the left had ceased. We may state at the outset that in the present investigation no such simple parallelism between the T wave and asynchronism of the end of ejection in the two ventricles was found, except when ventricular extrasystoles were induced, and even here the relationship was a qualitative one.

In this paper we shall first present the evidence for this statement and then discuss its significance in relation to other recent work in this field.

TECHNIQUE. The data were taken from aortic and pulmonary pressure curves recorded in anesthetized dogs by optical manometers simultaneously with leads II and III of the electrocardiogram, standardized in the usual manner. The technique of the operation and the various procedures employed were the same as those used before and already described (Katz, 1925; Wiggers, 1925), except that the pulmonary manometer was inserted directly through the arterial wall. A special flanged cannula was devised for this purpose. The two manometers were placed within

1 cm. of the semilunar valves in order to reduce differences in pulse transmission time to a minimum. The pericardium was not removed but an opening was made for the insertion of the pulmonary manometer, thus leaving the ventricles in their natural position and allowing them to contract in the normal manner.

Some five hundred and sixty of the best records, procured under normal control and various modifications of the circulatory conditions, were projected and transcribed on coördinate paper. A correction was made for the parallax of the electrocardiogram. None was necessary for the two pressure curves as the double slit lamp was used (Katz and Baker, 1924).

The relation of the onset, *B*, summit, *S*, and end, *E*, of the T wave to the end of left, *L*, and right, *R*, ventricular ejection was established in each of these records. The point chosen for the end of ejection was the beginning of the incisura (see fig. 5). In addition the following intervals were also ascertained: the height of the T wave above (or below) the isoelectric line late in diastole; the duration of the entire T wave (*BE*, fig. 1), and the duration of rising, *BS*, and of subsiding potential, *SE*. The eleven measurements of each of these records were tabulated and on this tabulation our report is based.

EXPERIMENTAL RESULTS. *A. Errors in measurement.* It is necessary in considering the data to establish first of all the accuracy of the measurements. There are several possible sources of error: 1, errors arising in establishing the various points on the T wave and the end points of left and right ventricular ejection; 2, errors resulting from differences in the sensitivity of the manometers and galvanometer string employed; and 3, errors arising from differences in transmission time in the aorta and pulmonary artery.

The accuracy of the actual measurement was ensured by our method of projecting a magnified image onto cross-section paper. In this manner the end of ejection could be obtained in the projected arterial pressure curves within 0.003 of a second, as shown by checking one another's independent measurements. The onset of the T wave is not always clearly defined, and sometimes when the galvanometer string is not at rest after the QRS complex, the point chosen is a matter of opinion. In such cases a note to this effect was made and the measurement was discarded. In most of our records the onset could be determined within 0.005 of a second. The summit of the T wave was readily measured in most curves. In some, where a plateau existed, the mid-point of the top was chosen. In diphasic T waves the time relations of both the positive and negative summits were measured. The end of the T wave is the sharpest point on the record and could be determined within 0.003 of a second. In all of these records the points selected were on the bottom of the curves.

The vibration frequency of the galvanometer string was so high that the error it introduced was negligible. Manometers with vibration frequencies of 150 double vibrations per second were used to record the pulmonary pressure, and a maximum error of 0.002 of a second might have been introduced. At the point where the manometers were inserted, the error arising from a difference in the transmission rate of the pulse wave in the aortic and pulmonary arteries (probably amounting to 7 meters per second) would have been, at most, 0.002 of a second. After a consideration of all these possible errors, we are convinced that the average error of any measurement in the present series is, at most, 0.012 of a second. However, the relation of the end of the T wave to the end of right or left ejection and the asynchronism of the ends of the two ejections, could be established within 0.01 of a second. This error of measurement was considered in interpreting our results, no significance being attached to changes unless the variations were more than this.

It is impossible to reproduce and treat in detail all the data which we accumulated for this report. Instead, typical figures, accurately retraced, and original curves are used to elucidate the facts.

B. Control experiments. The data obtained from eleven animals immediately after the apparatus was aligned, which we have called our control experiments, are graphically given in figure 1. The T waves transcribed in this figure are all from lead II. Although the time relations were not the same, no essential difference in interpretation was found between analyses based on lead II and those based on lead III, in the experiments where both leads were recorded. The latter are, therefore, omitted. The curves in figure 1 are all drawn to the same abscissal (time) and ordinate (millivolt) scale. The same ordinate values were assured by retracing the standardized curves at the same magnification. The same abscissal values for the tracings were secured by multiplying the abscissal values of the original curve by a factor A_s/A_o; where A_o is the abscissal value of the original curve and A_s of the standard curve (figure 1.) The abscissal and ordinate values of points, B, S and E were established, using S arbitrarily as the zero point. The curve was then drawn in after marking the ordinate and abscissal values of a few more points on the electrocardiogram. The time relations of the end of right and left ventricular ejection to the T wave were established in a similar manner and are shown by the end of the horizontal blocks, L and R. The beginning of these blocks is jagged as the asynchronism at the onset was not determined.

Examination of the curves so retraced in figure 1 shows clearly that there is no consistent relation between the degree of asynchronism and any of the variants of the T wave, such as its amplitude, duration, or even its direction. The lack of parallelism between the asynchronism and the

Fig. 1. Accurately retraced T wave (about 1½ times natural size) of eleven animals recorded under control conditions illustrating their relation to the ends of right and left ejection. Manner of construction described in text. Abscissae in seconds; ordinates, millivolts. *B* marks onset, *S* the summit, and *E* the end of the T wave. The right ends of the horizontal blocks, *L* and *R*, denote respectively the time relations of the end of left and right ejection. The numbers at the right of chart give the experiment number.

duration of rising and declining voltage is also apparent. For example, out of five experiments where there is no asynchronism,[1] two (55 and 57) have an inverted T wave, two (58 and 347) an upright T wave of short duration, and one (345) an upright T wave of long duration. In the other six experiments, right ejection outlasts left and in all of these the T wave is upright, but the amplitude and duration are in no way proportional to the degree of asynchronism. For example, in experiment 62, a large asynchronism is associated with a short T wave while in experiment 59 a smaller degree of asynchronism is associated with a larger and a longer T wave. Further discrepancies will be found on examining this figure, and thus the idea that the T wave is normally related, in a simple fashion, to the asynchronous ending of ejection in the two ventricles is at once eliminated.

Analysis failed to reveal any direct or inverse proportionality between the height of R and the height of T in this series. We were able to find in this small group four exceptions (36 per cent) to the statement made by Lewis (1925) that in general the end deflection and last initial deflection have an opposite sign. Thus, both experiments 55 and 57 had an S deflection and an inverted T wave, while in experiment 53 and 344 an upright T wave occurred in the absence of an S wave. No consistent relationship could be found between the T wave and any of the variants of the cardiac cycle, e.g., cycle length, Q-T interval, S-T interval, and the duration of ejection in either ventricle.

A glance at figure 1 shows that the T wave begins before the end of ejection in every case. In all but one instance (345) the ends of the ejections coincide or come after the summit, *S*, of the T wave. In six instances (55, 57, 53, 61, 347 and 344) the ends of both ejections occur during the decline of voltage; in one instance (60), left ejection ends during the decline of voltage and the right after the string has become stationary. In the other three experiments (58, 59 and 62) the ends of both ejections come after the T wave. There is, therefore, no better constancy in the relation of the T wave to the end of right ejection than to the left.

C. The effect of altering experimental conditions.[2] The following group of experiments is presented in order to show the absence of any constant relationship between the degree of asynchronism and the nature of the T wave in the same animal when the dynamic conditions of the circulation or the nature of the blood have been altered.

Figures 2, 3, 4, 6, 7 and 8 show this fact concisely. These figures consist of accurately retraced electrocardiographic curves (lead II) taken from

[1] For simplicity in presentation "asynchronism" in this paper is used to designate the asynchronism of the ends of right and left ventricular ejections unless otherwise amplified.

[2] The vagi were cut in these experiments.

original curves of each type of experimental alteration. The figures are constructed in the same manner as figure 1 except that the entire electrocardiogram (lead II) is given, and that the beginning of Q is taken as the zero point. The lowest curve in each figure is the control taken before the conditions were changed. In certain of the figures the amount of the modifications in the diastolic pressure level in the aorta and pulmonary artery, as related to that existing in the control, is illustrated by the length of the horizontal lines, *P* for the pulmonary, and *A* for the aortic pressure curves.

1. Increasing venous return. The effect of augmenting the venous return in experiment 60 is shown in figure 2. Saline infusion in this experiment

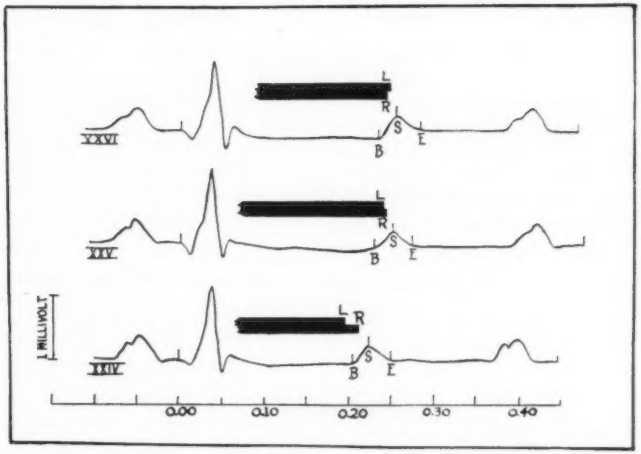

Fig. 2. Accurately retraced electrocardiograms, lead II (about natural size) recorded during saline infusion. Manner of construction and lettering of this and the following figures is similar to figure 1. Curve XXIV was recorded before the infusion; curves XXV and XXVI at various stages during the infusion.

increased the cycle length (Q to the end of the electrocardiographic tracing), and the Q-T interval. In the control record (XXIV) the ejection of the left ventricle ended before the T wave began and the end of the right ejection ceased while the electrical potential was rising. The infusion of saline caused both right and left ejection to continue for a longer time in regard to the T wave, the effect being more marked on the left ventricle in this particular experiment. As a result, the asynchronism decreased and later became reversed. There was, however, no appreciable change in the T wave accompanying this alteration in asynchronism. In other experiments a similar lack of parallelism was found, e.g., experiment 59 had no change in asynchronism while the T became broader and more upright,

and in experiment 61 the asynchronism decreased so that right ejection ended relatively earlier and yet the inverted T wave of the control became

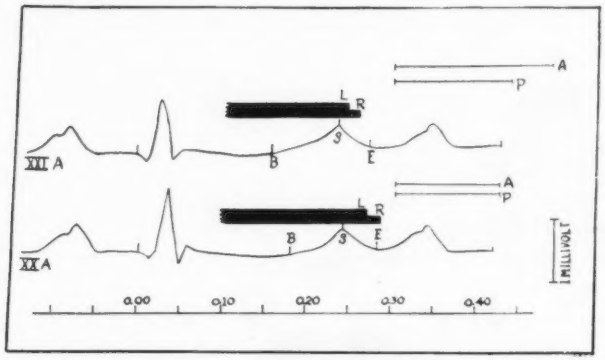

Fig. 3. Accurately retraced electrocardiograms, lead II (about natural size) recorded before and during compression of the thoracic aorta low down. Curve XX-*A* recorded before compression; curve XXI-*A*, during the compression. The horizontal lines *P* and *A* in this and the following figures denote the height of the diastolic pressures in the pulmonary artery and aorta as related to these values in the control record.

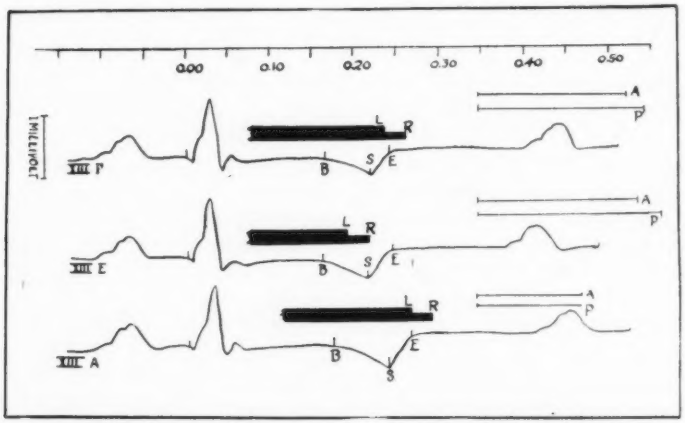

Fig. 4. Accurately retraced electrocardiogram, lead II (about natural size) recorded during the pressor influence of stimulation of the central end of the cut vagus. Curve XIII-*A* is the control record; curves XIII-*E* and -*F* were taken during and just after stimulation of the nerve.

upright. In some cases a parallelism was present, as might be expected on the laws of chance, e.g., in experiment 58 a diphasic T wave became in-

verted as the time interval by which the right ejection outlasted the left decreased.

The change in the time relation of the T wave to the end of both ejections in figure 2 is the reverse of the effect of saline infusion in most of the experiments. Here the T wave occurs earlier in relation to *L* and *R* in the record taken after infusion, than in the control, whereas in most of the others it occurs relatively later.

2. Increased arterial resistance. The increase in arterial resistance was accomplished either by compression of the thoracic aorta or by the pressor influence of stimulating the central end of the vagus. Figure 3 (expt. 59) shows a typical experiment with the former method, and figure 4 (expt. 55) one produced with the latter.

In figure 3, the increased height and duration of the upright T wave occurs without any change in asynchronism. In figure 4 the inverted T wave becomes shallower and shorter also without any change in asynchronism. Most of the other experiments in which the arterial pressure was raised, showed a similar lack of parallelism between the asynchronism and the T wave, although differing in the details of the changes.

Increasing the arterial resistance caused a decrease in the duration of ejection and of the Q-T interval but the amount of shortening was not equivalent in most of the experiments in this group; the effect on mechanical systole was greater so that after compression the T wave occurred later in relation to the ends of ejection as in figures 3 and 4. In a few instances the effect was just the reverse.

3. The effect of slowing the heart rate by vagus stimulation. All the experiments in which the heart was slowed by vagus stimulation showed a longer T wave; in some the height was increased, in others decreased, and in still others unchanged. Accompanying these alterations four experiments showed an increasing asynchronism, i.e., the time interval by which the right ejection outlasted the left was increased. In the other eight, however, no change in asynchronism was noted. The original curves of one of the experiments (53) are shown in figure 5.[3] In this particular case the asynchronism during vagus stimulation (segment A) is practically the same as that some time after the cessation of stimulation (C), and yet the increased size of the T wave in the former is apparent at a glance. Record B was taken immediately after the cessation of stimulation and at this time the T wave is still larger and the asynchronism visibly increased.

This record shows also the prolongation of both ejection and the Q-T

[3] The original curves are introduced at this time especially to show the sharpness of the point selected to mark the end of ejection. A comparison of these records with the retraced curves of other experiments will clarify the construction of the latter.

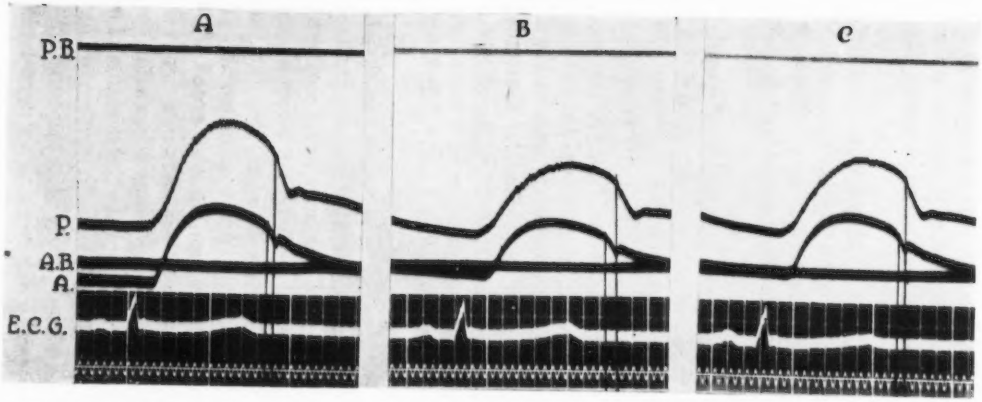

Fig. 5. Pulmonary and aortic pressure curve simultaneously recorded with the electrocardiogram lead II during stimulation of the peripheral end of the right vagus (about ½ natural size). Segment *A*, recorded during stimulation; *B*, immediately after the cessation of stimulation; *C*, some time later. *P. B.* is the base line for the pulmonary pressure curve *P*, and *A. B.* for the aortic pressure curve, *A*. *E. C. G.* is lead II of the electrocardiogram. The vertical black lines are drawn in to show the asynchronism of the end of right and left ventricular ejection. Time shown by the tuning fork line at the bottom; each double vibration is equal to 0.02 of a second.

interval. In this particular case, as in many of the other experiments in this series, the effect is more marked on ejection, as shown by its relatively later occurrence in respect to the T wave in record A and especially B as compared with C.

4. Alterations in CO_2. In several instances the effect of changes in blood CO_2 was studied. The CO_2 content of the blood was altered by varying the CO_2 tension of the inspired air, the tension of CO_2 in the

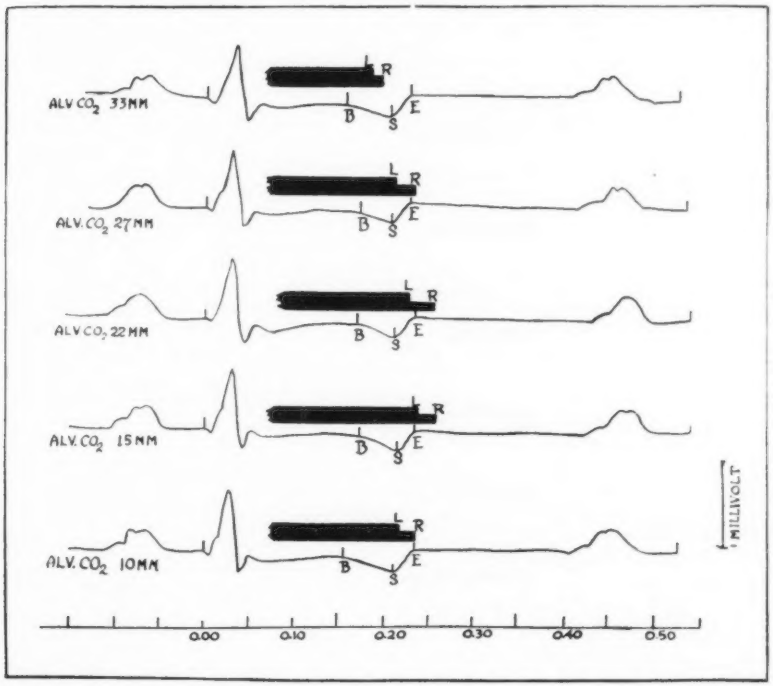

Fig. 6. Accurately retraced electrocardiograms, lead II (about natural size) recorded at various alveolar CO_2 tensions. Constructed as figure 1. The CO_2 tension in the alveoli at the time when the record was taken is indicated below each curve.

alveoli being measured by Mariott tubes (Banus, 1926). Figure 6 is made from records of such an experiment. In this instance the inverted T wave, present when the alveolar CO_2 tension was 10 mm., becomes shallower and shorter as the CO_2 tension increases, but at a CO_2 tension of 33 mm., it again becomes slightly broader. The asynchronism at first increases as the CO_2 rises, but later again decreases. Thus, in this case there is no parallelism between the changes in the T wave and in the asynchronism.

In this experiment the duration of the two ejections is at first lengthened and later shortened as the CO_2 increases. The Q-T interval, however, remains unchanged at first although later it shortens. The relation of the T wave to the end of both ejections is very variable, depending on the greater effect of CO_2 changes on mechanical ejection. Similar variations were observed in the other experiments of this group.

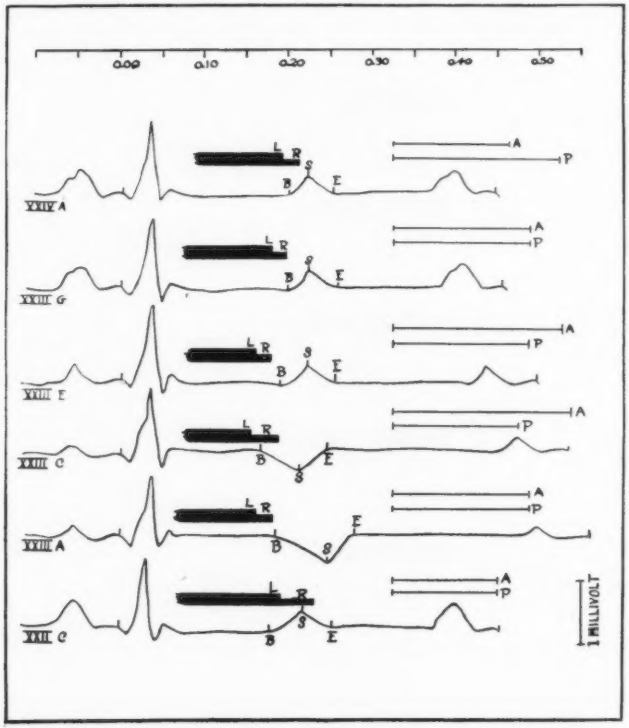

Fig. 7. Accurately retraced electrocardiograms, lead II (about natural size) recorded at various intervals after the intravenous injection of epinephrin. Constructed as figure 1. Curve XXII-*C* is the control; curves XXIII-*A*, -*C*, -*E*, -*G* and XXIV-*A* were recorded at various intervals after the injection of epinephrin.

5. Intravenous injection of epinephrin. The most striking changes in the T wave found in this investigation, other than with extrasystoles, occurred when epinephrin (2 cc. of 1/100,000 dilution) was injected intravenously. In all nine experiments the T wave tended to become "negative" in the sense that an upright T wave became smaller or inverted, and an inverted T wave became deeper. The asynchronism decreased in one instance only, in the others it remained unchanged or even increased.

The lack of parallelism between asynchronism and T wave changes is well illustrated in figure 7. The T wave, upright in the control (XXII C), becomes inverted soon after epinephrin is injected, and even before the abbreviation[4] and maximum pressure rise in the arteries occur. The inversion of the T wave is associated with a marked decrease in asynchronism. This parallelism between the change in the T wave and asynchronism does not hold later, for the lessened asynchronism persists even after the T wave has again become upright (cf. curves XXIII *E*, *G*, XXIV *A* of this figure).

It is interesting to note that in this particular experiment the marked abbreviation in the two ejections is accompanied by an increase in the Q-T interval producing very striking changes in the relation between the T wave and the end of ejection (compare XXII *C* and XXIII *A*). The relatively earlier occurrence of the end of ejection as regards the T wave present in this experiment was a uniform finding in all the experiments

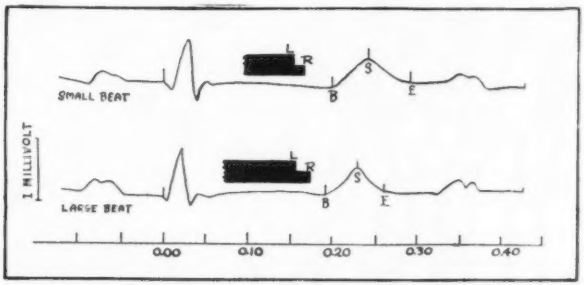

Fig. 8. Accurately retraced electrocardiograms, lead II (about natural size) of consecutive large and small beat of ventricular alternation induced by an excessive dose of epinephrin. Constructed as figure 1.

with epinephrin. In most of them, however, no initial prolongation of Q-T was observed.

6. Alternation. Many instances of post-extrasystolic alternation of the ventricles were observed. A comparison of the large and small beats failed to reveal any parallelism between the difference in the degree of asynchronism and the difference in the T wave of the two types of beats. In figure 8 the electrocardiogram of the small and large beat of an alternation, induced by the injection of an excessive dose of epinephrin, is given to illustrate this point. While the T wave of the large beat in this figure is longer and taller than that of the smaller beat, the asynchronism is reduced.

Figure 8 shows that the ends of both ejections occur relatively earlier

[4] The initial slowing is not due to a reflex through the vagus as both vagi were cut.

as regards the T wave in the small beat than in the large, the effect on the duration of mechanical ejection being greater than on the Q-T interval.

D. Ventricular extrasystoles. In striking contrast to the inconsistent relationship between the changes in the T wave and asynchronism found when the experimental conditions were altered, is the fact that *when ventricular extrasystoles occur a parallelism is to be observed in the changes of the T wave and asynchronism.* This statement is based on the analysis of forty-four sets of experiments in which the extrasystole occurred spontaneously (2 cases), or was induced artificially in every fifth beat in a heart artificially driven through the auricle by means of a Lewis interruptor, in the manner described by Wiggers (1925) (method II). These extrasystoles were forced from four sites, 1, the apical; 2, the basal region of the left ventricle; 3, the apical, and 4, basal region of the right ventricle.[5] With this technique it was possible to obtain in succession the electrocardiographic tracings from leads II and III under practically identical conditions.

Forty of these forty-four experiments showed a qualitative parallelism between the changes in the T wave and asynchronism, i.e., the T wave became inverted or the depth of its inversion increased, when the end of right ejection occurred earlier in relation to the end of left, and the T wave became upright or its height increased when the reverse was true.

Analyzed from the point of stimulation the following tabulation may be made:

TYPE OF EXTRASYSTOLE	NATURAL	LEFT APEX	LEFT BASE	RIGHT APEX	RIGHT BASE	TOTAL
Change in asynchronism and in T wave parallel (as defined above)	2	14	12	4	8	40
Change in asynchronism and in T wave *not* parallel	0	0	3	1	0	4
Total experiments	2	14	15	5	8	44

The four exceptions occurred in extrasystoles of the right apex and left base and none was present in the left apex and right base extrasystoles.

[5] The initial main deflection was always upright when right ventricular extrasystoles were induced (apex in five, base in six animals) but was not always inverted in left ventricular extrasystoles. One out of ten animals had an upright main initial deflection in the extrasystoles of the left apex, and six out of ten had an upright main deflection in the extrasystoles originating in the left ventricular base.

In all but two instances the T wave was in the opposite direction to the main initial deflection. One of the exceptions occurred in the extrasystoles induced in the left basal region (348), and the other in extrasystoles of the right apex (61). In both, the initial main deflection was upright, the former with, and the latter without an S deflection.

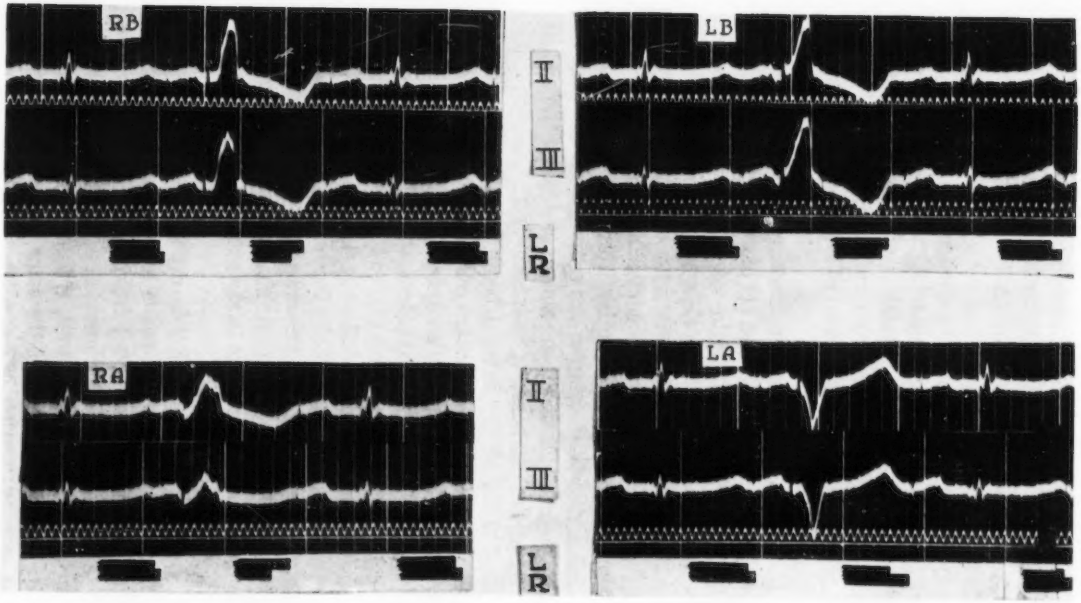

Fig. 9. Original electrocardiograms of an experiment in which extrasystoles starting in various regions of the ventricles were induced (about ½ natural size). In each segment lead II (labeled II) is superimposed over lead III (labeled III) using the onset of the preliminary vibration of the aortic pressure curve as the point of superposition. The pressure curves are omitted for convenience of reproduction. The time relation of the end of left and right ejection is accurately shown by the blocks *L* and *R* placed below each segment. In each segment the extrasystolic beat is shown along with the natural beat before and after it. The sharp deflection before the P wave is due to the shock which inaugurates each beat and the larger deflection preceding the extrasystole is produced by the shock causing the extrasystole. Segment *R. B.* shows the electrocardiographic tracings of an extrasystole starting in the basal region of the right ventricle; segment *L. B.*, an extrasystole starting in the basal region of the left ventricle; segment *R. A.*, an extrasystole starting in the apical region of the right ventricle; segment *L. A.*, an extrasystole starting in the apical region of the left ventricle. Time indicated by tuning fork record, each double vibration equalling 0.02 of a second.

In two of the four exceptions (348 and 58) the change in the T wave was not accompanied by any change in asynchronism, but in the other two the changes in asynchronism were in the opposite direction to that anticipated. These experiments were made under a variety of heart rates, venous inflows and arterial pressures.

While in practically all the experiments the changes in the T wave and asynchronism were in the same direction, this relationship was qualitative and not quantitative. This was true regardless of whether the asynchronism change was compared with the duration of the entire T wave, the period of rising electrical stress, the period of its subsidence, or with the change in height of the wave.

These facts may be made clearer by illustrative experiments. In figure 9 is shown the electrocardiogram leads II and III of extrasystoles, induced in the right base, *R.B.*, left base, *L.B.*, right apex, *R.A.*, and left apex, *L.A.*, in animal 62. In each instance lead II is superimposed over lead III, using the beginning of the preliminary vibration of the aortic curve as the time point for the superposition. For convenience of reproduction the pressure curves have been omitted from this figure and the time relations of the end of left and right ejection in the extrasystolic beat and in the natural beats before and following it are shown clearly by the end of the horizontal blocks, *L.* and *R.*, beneath each record.

The small upright T wave of the natural beats is associated with a slight asynchronism in which the right ejection outlasts the left, the asynchronism being slightly greater in the post compensatory beats. In three regions (*R.B.*, *R.A.* and *L.B.*), the induced extrasystoles have inverted T waves and *in each one the asynchronism is reversed so that now the left ejection outlasts the right.* In the fourth region, *L.A.*, the T wave becomes taller and *the asynchronism at the same time markedly increases.* The changes in the first three regions are not quantitatively related. Thus, the extrasystole originating in the right apex has the shallowest T wave of the three and yet the change in asynchronism is the greatest (compare *R.A.* with *L.B.* and *R.B.*).

A very interesting phenomenon occurred in experiment 58. On four occasions a series of two to four spontaneous extrasystoles of the left ventricle followed the extrasystoles forced from the right apex. The forced extrasystoles in this particular experiment were induced haphazardly at various times during diastole. A natural, a forced extrasystole, and two spontaneous extrasystoles with about the same duration of previous diastole are retraced over each other in figure 10. The natural beat in this experiment had a diphasic and the forced extrasystole of the right apex, an inverted T wave and yet in both beats the end of right and left ejection was synchronous. In the spontaneous extrasystoles from the left ventricle, two of which are shown in figure 10, the T wave became

upright and at the same time an asynchronism was developed in which the right ventricle continued to eject blood after the left had ceased. Thus, in the same record we have one type of extrasystole which forms an exception, one of the four previously mentioned, and another which like the majority follows the anticipated results.[6]

The change in the time relation between the T wave and the end of both ejections, when extrasystole occurs, that is the earlier occurrence of the

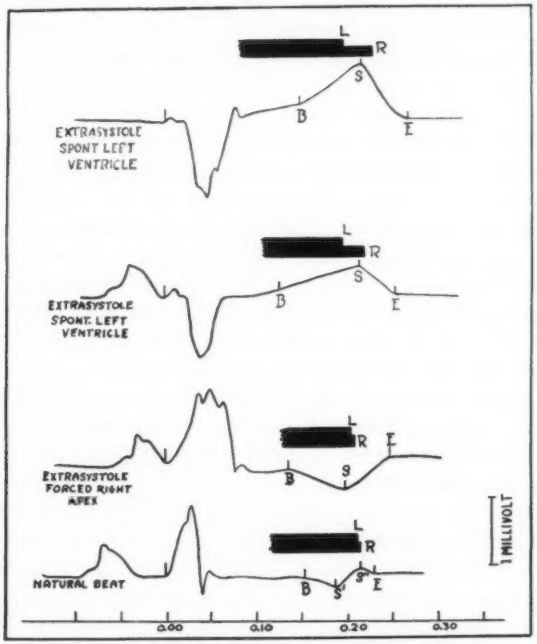

Fig. 10. Accurately retraced electrocardiograms, lead II (about natural size) taken from a single record in which natural beats, forced right apical extrasystoles and spontaneous left ventricular extrasystoles occurred in succession. The four selected curves are so labeled. Construction as in figure 1.

former in respect to the latter, is typical of the change in most experiments and proves again that when experimental conditions are altered the mechanical systole (ejection) is, as a rule, affected more than the electrical (Q-T).

[6] The middle two curves of figure 10 have a P wave just preceding the main deflection. The impulse from the auricle does not get through to the ventricle in these cases as such a short *PR* interval (one-half the normal of this animal) would be unlikely.

SIGNIFICANCE OF THE RESULTS. Obviously, we are not yet in a position to *fully* account for the occurrence and the changes in contour of the T wave. In clarifying the present status of our conception, it may be helpful to attempt a correlation of these results with the trend of recent investigations.

1. The validity of the T wave as the end of electrical disturbances. Recent work has shown that the time course of the rise and fall of electrical stress, in any region of the heart, can be obtained in cold-blooded animals as a monophasic action current, provided proper care is exercised as to the size of the electrodes and the amount of tissue intervening between the electrode on the injured and uninjured part of the ventricle (Adrian, 1921; Schellong, 1926).

The algebraic sum of the monophasic action current, under each electrode, gives the diphasic action current or electrogram in the sense of Samojloff (Yoshida, 1926). The duration of the monophasic action current is not the same in all regions but its termination varies independently of the time of its initiation (Yoshida, 1926). The results of Garten and Sulze (1916) and of Clement (1912) with differential electrodes show that even in closely adjoining regions the termination of electrical stress is independent of the time of its initiation, and the results of Erfmann (1913) indicate that this also *applies to warm-blooded animals.*

The electrocardiogram, Schellong (1926) has proven, differs from electrograms in that it records the electrical stresses of *all* parts of the heart; the rise and subsidence of electrical stress in any locality plays as much of a part in forming the electrocardiogram as the pathway of its spread and retreat, confirming a similar opinion clearly expressed by Mines (1913). The T wave in the electrocardiogram may thus be regarded as a manifestation of the persistence of electrical stress in certain fractions of the heart, caused by differences in time of excitation, or by *differences in their duration.* In other words, the opinion of Burdon-Sanderson and Page (1880), Bayliss and Starling (1892) and Einthoven (1913), is restated and more sharply defined by the results of recent investigations.

The electrocardiogram is in reality a first differential quotient expressing the changes in the algebraic sum of electrical stresses in the heart from moment to moment, oriented in the direction of the lead. It is thus equivalent to a tachygram. The T wave from this point of view is a period of disturbed electrical stress. Its end means that a stable condition has arisen, either as a result of cessation of electrical disturbances in all parts of the ventricles, or as a result of an accurate balance of changing stresses whose algebraic sum in the line of the lead remains zero. While it is improbable that large changes in electrical disturbances can be so nicely balanced for long periods of time, it is quite reasonable to expect smaller changes to be finely adjusted for short time intervals. In other

words, while the T wave may not actually mark the end of changes in electrical stress in the ventricles, it coincides reasonably well with this cessation.

2. Validity of the beginning of the incisura as the end point of ventricular contraction. The results of the present investigation led us to wonder whether the beginning of the incisura really marks the end of contraction in each ventricle, a tacit assumption which investigators in this field have made following the lead of Frank (1905). The evidence is strongly against such a conception.

In the first place, the end point is not simultaneous in the two ventricles, as one of us (Katz, 1925) first showed and as we have confirmed in this investigation. This asynchronism might be interpreted as indicating differences in the contraction time of the two ventricles, or—more probably—as due to different summations of the contractions of various fractions of the ventricles.[7]

Even if we admit for the moment that the duration of contraction of the fractions is the same in all parts of the heart, which we will show is not true, the work of Lewis and Rothschild (1915), recently confirmed by Wiggers (1927), has shown—contrary to Garten and his school (1916)—that the excitation of the various fractions of the surface of the heart is not simultaneous. From this point of view any end point selected can only be an approximation of the end of contraction. Relaxation of certain parts of the ventricle has probably begun before this arbitrary time limit; and contraction very likely continues in certain parts after this limit.

The incisura thus marks the cessation of the ejection of blood, or in other words, it marks the time when the ventricle, *as a unit*, ceases to contract and begins to relax, it does not mean that contraction is over in all parts of the ventricle.[8] Considered in the light of individual fractions we may say that the ventricle, *as a unit*, contracts as long as

$$\Sigma\, dx \cdot Cm > \Sigma\, dy \cdot Rm$$

and that it relaxes *as a unit* when

$$\Sigma\, dx \cdot Cm < \Sigma\, dy \cdot Rm,$$

where $\Sigma\, dx$ is the number of fractions of the ventricle contracting at the moment considered, $\Sigma\, dy$ the number of fractions relaxing at this moment, and Cm and Rm are respectively the mean effective velocity of contraction and relaxation of these fractions at that moment.

[7] Fractions denote a small section of cardiac muscle, and the unit may be chosen equivalent to one fibre, several fibres, or part of a fibre, without altering the idea, because the heart is a syncytium.

[8] The decisive alteration in the smooth contour of the pressure curve produced by the incisura precludes the idea that it is due to a progressively more rapid outflow of blood from the arterial system.

The beginning of the incisura marks, therefore, the point when

$$\Sigma\, dx \cdot Cm = \Sigma\, dy \cdot Rm.$$

Such an analysis might be superfluous were we considering the heart as a dynamic pump, but it is of the utmost importance when correlating mechanical with electrical activity.

Bearing these facts in mind, it becomes clear why no consistent time relation exists between the T wave and the points marking the end of right and left ventricular ejection.

3. An aspect of the cause of the T wave. If we grant that mechanical and electrical changes are manifestations of the same process, which we call excitation—and recent evidence points to such an interpretation (cf. Einthoven and Hugenholz, 1921; Arbeiter, 1921; Schellong, 1926)—and if we allow for the lag between the two, due to the viscid nature of muscular tissue (cf. Fulton, 1925; Gasser and Hill, 1924), then the asynchronous ending of ejection in the two ventricles is itself proof that the duration of activity, mechanical and electrical, is not the same in all regions of the mammalian heart. The fact that the asynchronism at the end of right and left systole is independent of the asynchronism at the onset (Katz, 1925) proves that the non-uniform termination of activity is due as much to variations in duration of this activity in different regions, as to differences in the order of excitation. The manner in which the ends of the fractionate contractions summate, explains the alteration in the asynchronism of the end of right and left ejection. The manner in which the terminations of the fractionate electrical stresses summate, explains the character of the T wave.

On this basis, the T wave is not due to persistence of electrical stress in one whole ventricle or the other; it is not due to persistence of electrical stress in the whole base or apex; nor yet is it entirely dependent on the pathway of invasion as Wilson and Herrmann (1920) and Lewis (1925) postulate. The lack of parallelism between the changes in asynchronism and the T wave, under most conditions, can best be explained and correlated with the findings of Erfmann (1913) and with the variations in monophasic action currents, by the hypothesis that the duration of electrical stress, even in closely lying fractions, is not the same. The results of such algebraic summations simulate persistence of electrical stress in one or another region.

The parallelism between the changes in asynchronism and the T wave in ventricular extrasystoles—which is probably true also for bundle branch blocks—shows that when the difference in the time of excitation of various regions is large, the effect of this on the termination of activity overshadows the natural variation in the duration of activity in various regions. Under such circumstances the pathway of retreat tends to follow

the pathway of invasion, in the sense of Lewis. Even in extrasystoles, the first approximation of the electrical axes, which we computed from leads II and III, showed that the axis at the time of the summit of the T wave in lead II was not in the opposite direction to that of the summit of the initial deflection in this lead, but formed various angles with it.

Wiggers (1927) has recently found that the changes in the duration of mechanical systole, which he and Katz (1922) had shown follow changes in initial length, arterial resistance and the injection of epinephrin, are not due to modifications in the time at which surface regions of the heart are excited. These changes must, therefore, be due to variations in the duration of fractionate contractions. Wiggers (1927) assumed, for simplicity, that the duration of the fractionate contractions was the same in all parts of the ventricles. This, however, is probably not the case. The variations in duration of fractionate contractions which occur when conditions are altered, might be expected to be present in different regions, even in a single systole, inasmuch as 1, the initial tension and arterial resistance are not the same in the two ventricles, nor vary in the same direction as conditions alter; 2, the direction of different heart muscle "fibres," even in adjacent regions, varies and consequently also their initial length and the resistance offered to shortening, and 3, the nutrition of different regions is variable. We believe these factors are sufficient to produce the variability in the length of time the fractions remain active.

SUMMARY

1. The time relation of the T wave to the end of ejection in the left and right ventricle was measured under a variety of experimental conditions. The measurements were made on records of aortic and pulmonary arterial pressure curves registered by optical manometers simultaneously with the standard leads II and III of the electrocardiogram. The curves were projected and transcribed on coördinate paper for measurement.

2. No parallelism existed between the asynchronism of the termination of ejection in the two ventricles and the character of the T wave under control conditions.

3. No consistent relation was found between the changes in the T wave and the variations in asynchronism of the end of right and left ejection when the circulatory conditions were altered. The T wave, on several occasions, became inverted without any accompanying change in this asynchronism.

4. The time relation of the T wave to the end of the two ejections varied considerably from animal to animal and in the same animal when conditions were altered. No better correlation could be made with the end of right ejection than with the left.

5. *A qualitative parallelism was found between the changes in asynchronism and the T wave when ventricular extrasystoles were induced.*

6. When correlated with the recent tendencies in the interpretation of electrical variations, the results indicate that the T wave is not due to a persistence of activity on one ventricle or another, nor to persistence at the apex or base, nor dependent on the pathway of invasion, but *rather to the non-uniform duration of activity in the various fractions of the ventricles.*

7. The electrocardiogram is in reality the first differential quotient (comparable to a tachygram) expressing the changes in the algebraic sum of electrical stresses in the heart from moment to moment, oriented in the direction of the lead. On this basis the T wave is the evidence of unstable electrical stress at the end of activity, produced by the non-synchronous cessation of electrical activity in all fractions of the heart.

8. The end point of ejection selected, namely; the beginning of the incisura in the arterial pressure curves, indicates the moment in each ventricle when the ventricle, *as a unit*, ceases to contract and begins to relax. It does not mark the cessation of all activity, some fibres having begun to relax before this period, and others continuing to contract after this time. The inconsistent relationship between the T wave and asynchronism of the end of right and left ejection is explained on this basis.[9]

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[9] Opening of the thorax and the insertion of arterial manometers does not disturb the electrocardiogram sufficiently to prevent the application of these results to the intact animal (cf. Katz, 1927).

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MUSCLE GLYCOGEN AS A SOURCE OF BLOOD SUGAR

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The carbohydrate which is present in the muscles as glycogen has been generally regarded as available for reconversion into blood sugar. Thus Jacobson (1920) stated that, in Eck fistula dogs, in which the portal blood-flow is diverted from the liver, the muscle tissue is apparently quite capable of effecting glycogenolysis as well as glycogenesis.

Collens, Shelling and Byron (1926) found in dogs in which the hepatic artery had been ligated, that the degree of hyperglycemia produced by adrenalin varied inversely with the period of time elapsing since the application of the ligature. Because no glycogen could be demonstrated in the tissues subsequent to hypoglycemic convulsions and death, they attributed their results to excessive carbohydrate oxidation involving complete utilization of all the glycogen stores of the body.

Recent experiments, however, have cast considerable doubt on the availability of the glycogen deposits in the muscles for replenishment of the falling blood sugar. Thus Best, Hoet and Marks (1926) caused severe insulin hypoglycemia in spinal cats, and found the glycogen content practically unchanged from its initial value in muscles not participating in the convulsions because of section of their nerves.

Pollman, Mann and Magath (1925) observed that decrease in muscle glycogen occurred concurrently with the development of hypoglycemia following removal of the liver from dogs, but that the hypoglycemic symptoms did not manifest themselves as rapidly in animals with a high initial muscle glycogen as in those in which this was low. Nevertheless, the relatively rapid development of hypoglycemic symptoms in such animals, in spite of the presence of appreciable amounts of glycogen in the muscles, led the investigators to believe that muscle glycogen cannot be broken down to glucose with sufficient rapidity to maintain the blood sugar at the normal level. The liver is therefore, they concluded, the prime factor in maintaining the normal level of glucose in the blood. The importance of the liver in this regard was further demonstrated in this laboratory by J. Markowitz (1925), who found that although hyperglycemia always fol-

[1] The expenses of this investigation were partly defrayed by a grant from the Carnegie Corporation.

lowed when epinephrin was repeatedly injected into fasting rabbits, this did not occur when measures were taken to rid the liver completely of glycogen. The muscles still contained some glycogen in several of the latter experiments.

In the present investigation, observations were made on the effects produced on the blood sugar of dogs, on which abdominal evisceration had been performed, by agents known to have a marked hyperglycemic action in the normal animal, such as epinephrin, ether anesthesia and asphyxia.

METHOD. In from eight to fourteen days after subtotal ligation of the vena cava, when the collateral veins in the abdominal parietes had become sufficiently enlarged, the animals were again anesthetised and the intestines, spleen, pancreas and liver removed, and the pedicles of the kidneys ligated. After recovery from this operation blood samples were taken at half-hourly intervals and such doses of adrenalin were injected, or such degrees of combined ether anesthesia and asphyxia were applied, as had been found adequate by previous experiments either on the same animal or on other normal dogs, to cause hyperglycemia. Adrenalin, administered subcutaneously in amounts equivalent to 0.1 cc. of 1/1000 solution per kilogram body weight, was found suitable and the desired degree of anesthesia and asphyxia was readily obtained by the administration of ether by the closed method, using a well-fitting gauze mask. When hypoglycemic symptoms appeared they were terminated by the injection of glucose, the experiment being continued during the decline of the artificially raised blood-sugar level.

RESULTS. The following protocols are typical of the results obtained. Graphs are presented for protocols 1 and 4 (figs. 1 and 2), those being the experiments in which the effects of epinephrin and asphyxia had been studied on the same animals both before and after evisceration.

Protocol 1. Dog 1 (see graph 1).

TIME		BLOOD SUGAR
		per cent
November 3, 1926	Normal. Weight 19.0 kgm. Last fed morning November 2	
3:30 p.m.	1.9 cc. of 1/1000 adrenalin chloride subcutaneously	0.113
4:00 p.m.	1.9 cc. of 1/1000 adrenalin chloride subcutaneously	0.121
4:30 p.m.	1.9 cc. of 1/1000 adrenalin chloride subcutaneously	0.132
5:00 p.m.		0.143
6:15 p.m.		0.192
7:00 p.m.		0.212
December 1, 1926	Subtotal ligation of vena cava, November 22, 1926 Weight 16.5 kgm. Last fed morning November 30	
2:15 p.m.		0.109
2:30 p.m.	Anesthetic started	
3:30 p.m.	Evisceration completed	0.098
3:45 p.m.	1.65 cc. of 1/1000 adrenalin chloride subcutaneously	
4:00 p.m.		0.090
4:15 p.m.	1.65 cc. of 1/1000 adrenalin chloride subcutaneously	
4:30 p.m.		0.065
4:45 p.m.	1.65 cc. of 1/1000 adrenalin chloride subcutaneously	
5:00 p.m.		0.056
5:30 p.m.		0.043
5:35 p.m.	Convulsions started	
5:40 p.m.	20 cc. of 10 per cent glucose intravenously 100 cc. of 10 per cent glucose subcutaneously Convulsions ceased immediately	
6:10 p.m.		0.087
6:40 p.m.		0.071
7:10 p.m.	1.65 cc. of 1/1000 adrenalin chloride subcutaneously	0.068
7:40 p.m.	1.65 cc. of 1/1000 adrenalin chloride subcutaneously	0.059
8:10 p.m.	1.65 cc. of 1/1000 adrenalin chloride subcutaneously	0.048
8:40 p.m.	Convulsions started as sample taken	0.037

Protocol 2. Dog 2.

TIME		BLOOD SUGAR
		per cent
November 16, 1926	Subtotal ligation of vena cava, November 4, 1926 Weight 12 kgm. Last fed morning November 15	
10:30 a.m.	Anesthetic started	
11:00 a.m.	Operation begun	0.135
12:00 n.	Evisceration completed	0.113
	1.2 cc. of 1/1000 adrenalin chloride subcutaneously	
12:30 p.m.	1.2 cc. of 1/1000 adrenalin chloride subcutaneously	0.124
1:00 p.m.	1.2 cc. of 1/1000 adrenalin chloride subcutaneously	0.089
1:30 p.m.		0.076
1:35 p.m.	Convulsions started	
2:00 p.m.	1.2 cc. of 1/1000 adrenalin chloride subcutaneously	0.070
2:10 p.m.	Died in convulsions	

Protocol 3. Dog 4.

TIME		BLOOD SUGAR
		per cent
December 3, 1926	Subtotal ligation of vena cava, November 25, 1926 Weight 6.2 kgm. Last fed morning December 2	
2:45 p.m.		0.089
2:55 p.m.	Anesthetic started	
3:55 p.m.	Evisceration completed	
4:00 p.m.	0.62 cc. of 1/1000 adrenalin chloride subcutaneously	0.091
4:25 p.m.	Almost asphyxiated by mucus in trachea Artificial respiration necessary to revive	
4:30 p.m.	0.62 cc. of 1/1000 adrenalin chloride subcutaneously	0.064
5:00 p.m.	0.62 cc. of 1/1000 adrenalin chloride subcutaneously	0.048
5:15 p.m.	Convulsions started	
5:20 p.m.	20 cc. of 10 per cent glucose intravenously 60 cc. of 10 per cent glucose subcutaneously	
5:25 p.m.	Attempts to get off table and walk about	
5:40 p.m.		0.129
6:10 p.m.		0.097
6:40 p.m.	0.62 cc. of 1/1000 adrenalin chloride subcutaneously	0.070
7:00 p.m.	Marked dyspnea	
7:10 p.m.	Died	

Protocol 4. Dog 5 (see graph 2).

TIME		BLOOD SUGAR
		per cent
December 7, 1926	Normal. Weight 12.5 kgm. Last fed morning December 6	
2:00 p.m.	Anesthetic begun	0.115
2:05 p.m.	Surgical anesthesia	0.172
2:30 p.m.	Anesthetic stopped	0.264
3:00 p.m.		0.194
3:30 p.m.	Anesthetic begun	0.125
3:40 p.m.	Anesthetic stopped	0.143
4:00 p.m.		0.133
4:30 p.m.		0.109
5:00 p.m.		0.106
December 29, 1926	Subtotal ligation of vena cava, December 13, 1926 Weight 11.0 kgm. Last fed morning December 28	
10:35 a.m.		0.111
10:40 a.m.	Anesthetic begun	
11:00 a.m.		0.132
11:05 a.m.	Operation begun	
11:45 a.m.	Evisceration completed. Anesthetic stopped	
11:55 a.m.		0.112
12:30 p.m.	Since last blood sample, has been partially asphyxiated several times by mucus in throat	0.087
1:00 p.m.		0.076
1:10 p.m.	Anesthetic begun	
1:25 p.m.	Anesthetic stopped	0.064
2:00 p.m.		0.054
2:10 p.m.	Convulsions started	
2:18 p.m.	5 cc. of 10 per cent glucose intravenously 15 cc. of 10 per cent glucose subcutaneously	
2:20 p.m.	Takes notice and wags tail when called	
2:45 p.m.		0.062
3:10 p.m.	Slight twitchings	
3:15 p.m.		0.054
3:25 p.m.	Anesthetic started	
3:40 p.m.	Anesthetic stopped	0.045
3:50 p.m.	Convulsions began 20 cc. of 10 per cent glucose intravenously 40 cc. of 10 per cent glucose subcutaneously	
4:00 p.m.	Convulsions ceased. Animal takes notice	
4:10 p.m.		0.102
4:40 p.m.		0.085
5:10 p.m.		0.070
5:15 p.m.	Anesthetic started	
5:30 p.m.	Anesthetic stopped	0.064
5:35 p.m.	Died	

Protocol 5. Dog 6.

TIME		BLOOD SUGAR
		per cent
December 9, 1926	Subtotal ligation of vena cava, November 25, 1926 Weight 9.5 kgm. Last fed morning December 8	
10:45 a.m.		0.091
10:50 a.m.	Anesthetic started	
11:05 a.m.		0.162
11:10 a.m.	Operation begun	
11:55 a.m.	Evisceration completed. Anesthetic stopped	0.169
12:25 p.m.		0.139
12:55 p.m.		0.121
1:00 p.m.	Anesthetic started	
1:15 p.m.	Anesthetic stopped	0.105
1:45 p.m.		0.096
2:15 p.m.		0.089
2:30 p.m.	Isolated tremors. Stopped taking notice	
2:45 p.m.		0.061
3:15 p.m.		0.059
3:25 p.m.	Marked nystagmus	
3:30 p.m.	20 cc. of 10 per cent glucose intravenously 60 cc. of 10 per cent glucose subcutaneously	
3:35 p.m.	Begins to take notice. Wags tail when called	
4:00 p.m.		0.116
4:30 p.m.		0.127
5:00 p.m.		0.121
5.05 p.m.	Anesthetic started	
5.20 p.m.	Anesthetic stopped	0.100
5.50 p.m.		0.108
6.20 p.m.		0.084

Protocol 6. Dog 7.

TIME		BLOOD SUGAR
		per cent
December 16, 1926	Subtotal ligation of vena cava, December 2, 1926 Weight 7.5 kgm. Last fed morning December 15	
11:25 a.m.		0.111
11:30 a.m.	Anesthetic started	
11:40 a.m.		0.127
11:50 a.m.	Operation begun	
12:40 p.m.	Evisceration completed. Anesthetic stopped	0.149
1:10 p.m.		0.138
1:40 p.m.		0.116
2:00 p.m.	Anesthetic started	
2:15 p.m.	Anesthetic stopped	0.079
2:45 p.m.	Almost asphyxiated by mucus in trachea	0.067
3:15 p.m.	Convulsion began 20 cc. of 10 per cent glucose intravenously 40 cc. of 10 per cent glucose subcutaneously	
3:20 p.m.	Begins to take notice	
3:45 p.m.		0.157
4:15 p.m.		0.111
4:45 p.m.		0.085
4:50 p.m.	Anesthetic started	
5:05 p.m.	Anesthetic stopped	0.082
5:45 p.m.		0.048
6:00 p.m.	Ceased taking notice	
6:15 p.m.		0.042

DISCUSSION AND SUMMARY. Confirming previous investigations, evisceration is shown to cause a rapid and progressive decline of the blood-sugar level in dogs, and in this condition, adrenalin, ether anesthesia and asphyxia exert no apparent influence on the blood-sugar level. These results, contrasted with the marked hyperglycemic effects of these agents in the normal animal (as shown in protocols 1 and 4), are interpreted as showing that the liver is the sole source of supply of blood sugar. This indicates that the sugar of the blood, once deposited as glycogen in the muscles, cannot be returned as sugar to the blood. Once it has been deposited as glycogen in the muscles, glucose may be considered to have entered an irreversible reaction and cannot again appear as blood sugar in the absence of the liver. Its only pathway is through lactic acid. If this is produced in excess, some may enter the blood stream and in an intact animal may be converted into glycogen in the liver. The depletion of muscle glycogen in eviscerated animals, noted by previous workers, may be accounted for by its gradual utilization on account of muscular activity, this effect being greatly accelerated by the onset of hypoglycemic convul-

sions. The slower rate of fall, or even the slight rise of blood sugar observed at the outset of the foregoing experiments is, of course, due to the effect of the anesthetic administered before the evisceration had been completed.

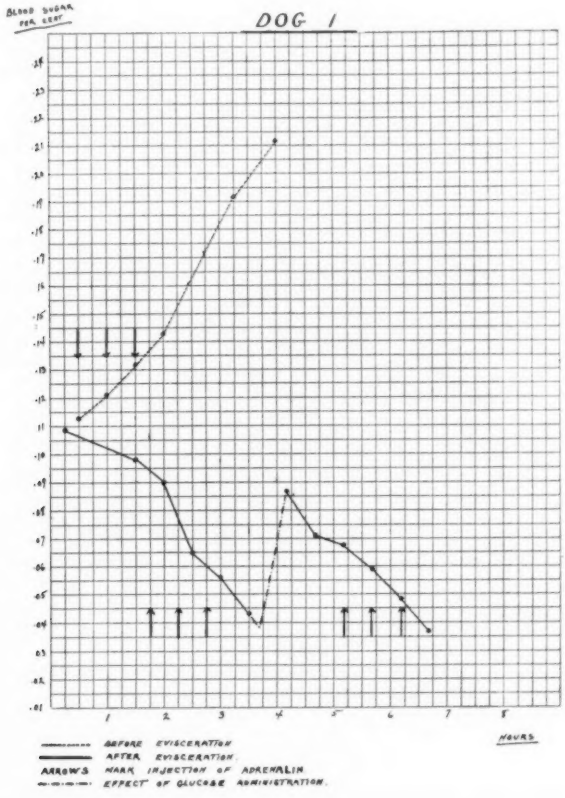

Fig. 1. Curves showing the effect on the blood sugar (ordinates) produced by adrenalin injected subcutaneously at the time intervals (abscissa) indicated by the arrows. The curves are from the same animal, the upper one before and the lower one after evisceration. Glucose had to be injected during the course of the evisceration experiment to restore the blood sugar.

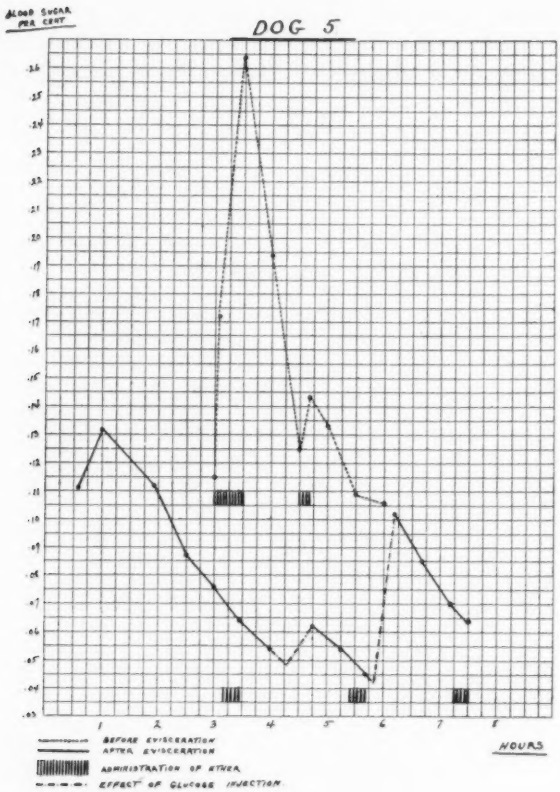

Fig. 2. Curves showing the effect on the blood sugar produced by ether anesthesia coupled with asphyxia before and after evisceration. For further details see figure 1.

CONCLUSIONS

1. Adrenalin, ether anesthesia and asphyxia have no effect on the falling blood-sugar level of abdominally eviscerated dogs.

2. Muscle glycogen is not an available source of blood sugar in the absence of the liver.

3. The liver, in the absence of food, is therefore the sole source of supply for the glucose in the blood.

I wish to take this opportunity of expressing my appreciation for the direction of Prof. J. J. R. Macleod, and the kindly interest of Dr. W. R. Campbell.

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THE PREVENTION OF TETANY BY ORAL ADMINISTRATION OF MAGNESIUM LACTATE

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Stimulated by the investigations of MacCallum and Voegtlin (1909) on the relation of parathyroid tetany to calcium metabolism, Berkeley and Beebe (1909) made a detailed study of the effect of salts other than calcium upon the tetany syndrome. They were able to demonstrate that intravenous injections of strontium and magnesium following bleeding were effective in causing the disappearance of the violent neuro-muscular symptoms, and furthermore, that coincident with the alleviation of symptoms there occurs a marked decrease in electrical excitability of the peripheral nerves. On the basis of their work they stated that since strontium and magnesium, when injected, temporarily relieved tetany, and since both salts appeared to be as efficacious as calcium, the latter could hardly be said to be specific in the treatment of tetany. Magnesium, they stated, is a powerful depressant and is dangerous to life when given in a dose sufficient to control the symptoms of tetany.

In 1911 Voegtlin and MacCallum, working on the influence of various salts upon tetany, found that the symptoms of tetany can be temporarily relieved by intravenous injections of magnesium, thus confirming Berkeley and Beebe's work. They showed that magnesium causes a decrease in the electrical excitability of the motor nerves and brings about a general state of anesthesia in both normal dogs and dogs in tetany. Although all tetany symptoms disappeared following intravenous injections of magnesium salts, Voegtlin and MacCallum were unable to keep the dogs alive more than seven days—the animals invariably succumbing to tetany.

Previous work by the writer (Swingle and Wenner, 1926) had demonstrated that by continuous oral administration of strontium, parathyroidectomized dogs can be kept free from tetany for long periods and if treated sufficiently long may eventually permanently recover.

The present work was undertaken with the object of determining whether or not magnesium salts are likewise efficacious in preventing tetany and if so what is the mechanism of their action in relation to blood calcium and tetany prevention. Survey of the literature, and previous work (Swingle and Wenner, 1926) on the effect of bleeding upon the alleviation of tetany,

had convinced the writer that if magnesium salts are effective in the treatment of tetany, they evidently exert some influence upon calcium metabolism. It was considered unlikely that magnesium *per se* could substitute for calcium. With this object in view the following experiment was undertaken.

The writer takes this opportunity of acknowledging his gratitude to Prof. W. W. Swingle for suggesting the problem, and for his untiring interest and kindly criticism throughout the progress of the work.

Ten dogs were used in this investigation. One grain of morphine sulphate and 1/150 grain atropine sulphate dissolved in 2 cc. of sterile water were injected one hour or more before the operation. This procedure is very beneficial for morphine causes the dog to take ether well and atropine prevents salivation. All parathyroids were identified before the thyroids and surrounding fascia were removed. After excision, the thyroids and connective tissue were again examined and the parathyroids again identified. It is quite evident that by this method all parathyroids were removed. All dogs developed tetany some time after operation; those dogs that did not show tetany symptoms within the usual period were fed large quantities of meat, and tetany followed within eighteen hours.

The animals were kept on a modification of the diet used by Cowgill (1923) for metabolism experiments. The diet mixture consisted of casein 38.8 per cent, sucrose 35.3 per cent, lard 17.6 per cent, butter 7.8 per cent, and salt mixture (sodium chloride 10 grams, ferric citrate 4 grams and few drops of Lugol's solution) 0.6 per cent (Swingle and Rhinhold, 1925). Approximately 16 grams of this mixture per kilo body weight were fed the animals daily. Following the onset of tetany, when the dogs refused food, warm water was added and the diet mixture administered by stomach tube. The use of this diet does not delay in any way the onset of tetany in dogs. This fact has been adequately demonstrated by several investigators (Inouye, 1924) (Swingle and Wenner, 1926).

Magnesium lactate in 5 per cent solution was given by stomach tube three times daily in 100 cc. doses. This amount was decreased at times for occasionally one of the dogs would become stuporous due to the anesthetic effect of the magnesium. The dosage was lessened also at later periods when the experiment was drawing to a close. The medication began in all cases except dog 10, a few hours after operation, when the animals had recovered from the effects of the anesthetics.

Milk was added to the diet of those dogs that showed marked tetany symptoms for several consecutive days. Magnesium lactate given at this time did not bring about an increase in the calcium content of the serum, but, through its depressing action on the central nervous system, concealed the more violent symptoms of tetany. The rise in calcium was obtained

by giving one-half pint of milk along with magnesium. Although milk contains only 1.2 grams of calcium per liter, one-half pint given daily for a few days is sufficient to bring the serum calcium above the tetany level when administered along with magnesium. When this new calcium level was attained milk was discontinued. Further administration of magnesium lactate tended to keep the serum calcium at this point. Dog 3 remained in tetany from the third to the tenth day. The animal received one-half pint of milk daily from the eighth to the eleventh day. Tetany disappeared on the tenth day and on the twelfth day, when the milk was discontinued, the serum calcium was 8.1 mgm. per 100 cc. Dogs 3, 7, 8, 9, 10 received milk whereas milk was not given to the remaining five. The data of two of the ten experimental dogs are summarized in table 1.

Serum calcium was determined at the time of operation and at varying intervals thereafter. Clark and Collip's (1925) modification of Kramer and Tisdall's (1921) method for serum calcium determination was used. Blood was aspirated directly from the left ventricle of the heart. This method of drawing blood was employed because of its simplicity. The withdrawal of large quantities of blood is to be avoided for following hemorrhage the serum calcium level is elevated (Swingle and Wenner, 1926) and the pH falls (Bennett, 1926). Since 20 cc. of dogs' blood will produce only 6 to 7 cc. of serum, calcium determinations were not made as often as might have been desired. Figures 1 and 2 show the plotted curves of the serum calcium of dogs 2 and 3.

Dog 2, figure 1, never showed symptoms of tetany up to the eighteenth day following operation. Serum calcium remained at about 8 mgm. per 100 cc. for twelve days. On the eighteenth day the dog was fed a large quantity of meat. Violent tetany occurred on the following day, serum calcium then was 6.1 mgm. Three hundred cubic centimeters of a 5 per cent solution of magnesium lactate were given. Five hours later tetany disappeared. The calcium on the twentieth day was 8.97 mgm. Tetany never again appeared. Magnesium therapy was discontinued on the twenty-eighth day, when the dog was placed on a meat diet without ill effects resulting. It is interesting to note that this dog received no milk.

From the results obtained with strontium lactate and magnesium lactate it was considered probable that the lactates of other cations would be as effective if the dosage could be carefully regulated. With this idea in mind, sodium and potassium lactate were tried. Cadmium chloride was also used. However, cadmium chloride and cadmium and sodium lactate proved to be so irritating to the gastro-intestinal tract that 100 cc. of $\frac{1}{2}$ of 1 per cent solution will cause the most violent vomiting. Potassium lactate produced no gastric disturbance but was practically inert as a therapeutic agent in the treatment of parathyroid tetany. Potassium lactate was administered to three parathyroidectomized dogs. Tetany

appeared, in all cases, in the usual time followed by death several days later. Potassium lactate in large amounts, 300 cc. of a 5 per cent solution, had no effect upon relieving tetany. These findings with potassium lactate agree with those obtained by Voegtlin and MacCallum (1911). Table 2

TABLE 1

DATE	TIME	SERUM CALCIUM	MAGNESIUM LACTATE GIVEN	REMARKS
Dog 7. Terrier ♂, 7 kilos				
1926		*mgm. per 100 cc.*		
Jan. 13	4:00 p.m.	10.7	300 cc. 5 per cent solution	Thyroparathyroidectomy
Jan. 14–17				Normal
Jan. 18	11:30 p.m.	5.9	300 cc. 5 per cent solution	Tetany
Jan. 19–20			300 cc. 5 per cent solution	No tetany
Jan. 21			300 cc. 5 per cent solution	Slight tremors chattering of teeth
Jan. 22–28			300 cc. 5 per cent solution	1 pint of milk given daily
Jan. 29		10.0	100 cc. 5 per cent solution	Milk discontinued
Feb. 4			100 cc. 5 per cent solution	Normal
Feb. 5		7.5	100 cc. 5 per cent solution	No tetany
Feb. 6–8			100 cc. 5 per cent solution	No tetany
Feb. 9	4:00 p.m.	6.2		Slight tetany. Muscular twitching. No magnesium lactate
Feb. 10	3:00 p.m.		5 gm. dissolved in ½ pt. of milk	Tetany
	9:00 p.m.		5 gm. dissolved in ½ pt. of milk	Slight improvement
Feb. 11			300 cc. 5 per cent solution given	Recovered, ½ pint milk
Feb. 12–17			100 cc. 5 per cent solution	Normal. No milk given
Feb. 18		7.6	100 cc. 5 per cent solution	Normal. No milk given
Feb. 19–24			100 cc. 5 per cent solution	No tetany
Feb. 25–26		7.6	50 cc. 5 per cent solution	No tetany

TABLE 1—Continued

DATE	TIME	SERUM CALCIUM	MAGNESIUM LACTATE GIVEN	REMARKS
		Dog 7. Terrier ♂, 7 kilos—*Continued*		
1920		*mgm. per 100 cc.*		
Feb. 27	8:30 a.m.		50 cc. 5 per cent solution	Tetany, ½ pint milk given with magnesium lactate
	2:30 p.m.			Recovered. Animal apparently normal
Feb. 28–Mar. 6			100 cc. 5 per cent solution	Normal
Mar. 7–8				No magnesium lactate given. Dog fed small portions of meat
Mar. 9		9.3		Normal
Mar. 10–18				Dog normal
Mar. 19	3:00 p.m.		300 cc. 5 per cent solution	Violent tetany. Enema given
	6:00 p.m.			Condition improved; ½ pint milk given
Mar. 20			300 cc. 5 per cent solution	Tetany continues. Twitching not so marked, 1 pint milk given
Mar. 21–22			200 cc. 5 per cent solution	Dog better. 1 pint milk given
Mar. 23–24			100 cc. 5 per cent solution	1 pint milk daily
Mar. 25–26				Dog normal. Fed 1 pint milk bread and small piece of meat
Mar. 27		10.1		Normal. Placed on meat diet. No magnesium lactate given
Mar. 28–Apr. 21				Normal
Apr. 22	11:30 a.m.			Tetany. Animal had not defecated for about 3 days. Enema given. No magnesium lactate administered
	4:30 p.m.			Tetany symptoms gone
Apr. 23–27				Normal
Apr. 28	3:45 p.m.	9.5		Normal (splenectomy)
June 10				Chloroformed

TABLE 1—*Concluded*

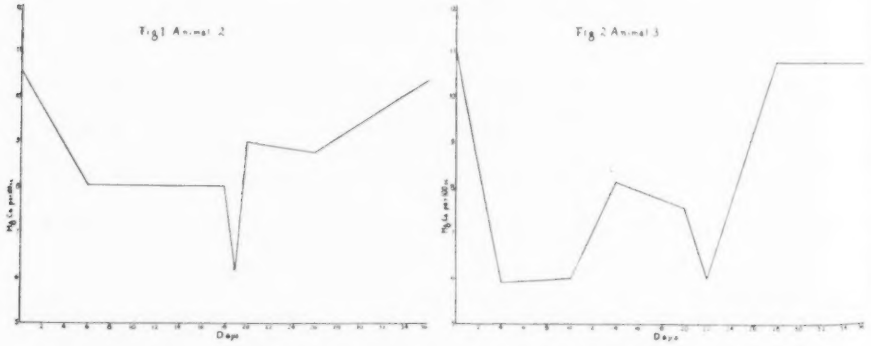
DATE	TIME	SERUM CALCIUM	MAGNESIUM LACTATE GIVEN	REMARKS
Dog 9. Mongrel ♂, 20 kilos				
1926		*mgm. per 100 cc.*		
Feb. 1		11.0		Thyroparathyroidectomy
Feb. 2–9			300 cc. 5 per cent solution	Normal
Feb. 10	10:30 a.m.		100 cc. 5 per cent solution	Tetany
	3:00 p.m.	6.5	100 cc. 5 per cent solution	Tetany very severe
	9:20 p.m.	6.3	100 cc. 5 per cent solution	Animal greatly improved. ½ pint milk given
Feb. 11–12			300 cc. 5 per cent solution	Normal, ½ pint milk given
Feb. 13–17			300 cc. 5 per cent solution	Animal is very inactive. No milk
Feb. 18–19			300 cc. 5 per cent solution	Very stuporous. Given 1 pint milk
Feb. 20–22			300 cc. 5 per cent solution	Slight muscle tremors
Feb. 23–24			300 cc. 5 per cent solution	No tetany. Animal stuporous
Feb. 25–Mar. 5			200 cc. 5 per cent solution	Dog normally active
Mar. 6		10.1		Magnesium therapy discontinued. Dog placed on meat diet
Mar. 7–24				Normal
Mar. 25		10.3		Normal
Mar. 26–Apr. 10				Slight tetany. Dog has not defecated for 2 days. Enema given. Tetany disappeared 3 hours later
Apr. 11–May 9				Normal
May 10		10.8		Normal
May 13				Normal. Used for other experiments

shows the ineffectiveness of cadmium, sodium and potassium in the treatment of tetany.

Six parathyroidectomized dogs were treated with cadmium lactate, one with cadmium chloride and two with sodium lactate. Both cadmium and sodium salts produced violent vomiting which hastened the appearance of

tetany symptoms. Vomiting causes a loss in acid and if long continued results in tetany possibly due to alkalosis. In this case the withdrawal of acid combined with the absence of the parathyroid hormone was responsible for the early onset of tetany. Typical tetany spasms occurred within thirty-six hours after operation and death followed, on the average, within three days. It was thought at the time that sodium lactate, if assimilated, would cause an increase in the sodium ion and bring on tetany much sooner. Although tetany did appear at a much earlier period it was not due to an increase in the sodium of the blood caused by the assimilation of sodium lactate for all of the sodium lactate given by stomach tube was vomited immediately.

The fatal results obtained by the use of cadmium, potassium and sodium serve very convincingly as proof that the results obtained by the use of



Figs. 1 and 2. Curves showing the effect of magnesium on the level of the serum calcium. Dog 3 received milk when the low points on the curve were reached Dog 2 received no milk.

magnesium could not be due to any failure in removing all parathyroid tissue at the time of operation since the writer performed all operations and used the same technique.

From the data presented here it would appear that magnesium in some way aids to some extent, other than through its depressant action, in rendering the calcium more soluble thus raising the content of this element in the blood. Greenwald (1925) has suggested that in the absence of the parathyroid hormone, calcium phosphate becomes less soluble and is precipitated in the tissues and in this way is removed from the blood stream rather than by elimination from the organism through the gut wall as proposed by Salvesen (1923). It may well be that a bivalent cation other than calcium, when in excess may unite with the phosphate and thus liberate the calcium in a more soluble form and place it in the

TABLE 2

The effect of lactates of cadmium, sodium and potassium

ANIMAL	DATE OF OPERATION	DATE TETANY FIRST APPEARED	LIFE SPAN	AMOUNT SALT GIVEN DAILY	REMARKS
				Cadmium lactate	
	1926		*days*		
1	Mar. 16	Mar. 17	3	100 cc. 2 per cent solution cadmium chloride	Salt caused violent vomiting and frothing at mouth
2	July 7	July 9	3	100 cc. 5 per cent solution cadmium lactate	Violent gastric disturbance caused by cadmium lactate. Animal died of typical tetany symptoms
3	July 7	July 10	4	100 cc. 1 per cent solution cadmium lactate	Violent gastric disturbance caused by cadmium lactate. Animal died of typical tetany symptoms
4	July 8	July 9	2	100 cc. 1 per cent solution cadmium lactate	Died in convulsions. Vomited entire amount of cadmium solution
5	July 10	July 12	2	100 cc. 0.5 per cent solution cadmium lactate	Died in convulsions. Vomited entire amount of cadmium solution
6	July 15	July 17	4	100 cc. 0.5 per cent solution cadmium lactate	Died in convulsions. Vomited entire amount of cadmium solution
7	July 15	July 17	2	100 cc. 0.5 per cent solution cadmium lactate	Died in convulsions. Vomited entire amount of cadmium solution
				Sodium and potassium lactate	
1	July 20	July 22	3	200 cc. 1 per cent solution sodium lactate	Dog vomited after every administration of sodium lactate. Died in convulsions
2	July 20	July 21	1	300 cc. 0.5 per cent solution sodium lactate	Dog vomited after receiving solution. Died in tetany
3	Oct. 4	Oct. 7	8	300 cc. 5 per cent solution potassium lactate	Solution retained. Tetany never abated once it appeared
4	Oct. 4	Oct. 8	4	300 cc. 5 per cent solution potassium lactate	Tetany appeared on the 4th day. Potassium lactate had no effect in relieving tetany
5	Oct. 5	Oct. 9	14	300 cc. 5 per cent solution potassium lactate	Tetany appeared on 4th day and continued until death occurred

blood. However, once animals develop tetany magnesium administration exerts little effect other than to disguise the more violent symptoms through its anesthetic action. The serum calcium when below the tetany level, can be raised in most cases only by administration of calcium in some available form. Milk is a very good source of calcium and for this reason was chosen for the purpose of increasing the serum calcium. When this rise in serum calcium is secured, continued magnesium therapy tends to maintain the calcium content of the serum at this new point.

Table 1 and figures 1 and 2 show that on the average, operated dogs receiving magnesium develop tetany at later periods than untreated dogs. It is well known that dogs receiving no medication show tetany symptoms within three or four days. Dog 10 received no magnesium following operation, developed tetany within twenty four hours, whereas all other

TABLE 3

Effect of magnesium in delaying onset of tetany

ANIMAL	DATE OPERATED	Ca NORMAL	DATE TETANY	Ca	INTERVAL OPERATION—TETANY
		mgm.		*mgm.*	*days*
1	Oct. 19	10.6	Oct. 24	5.8	5
2	Oct. 23	10.3	Nov. 11	6.1	19
3	Oct. 29	10.9	Nov. 2	5.8	4
4	Nov. 4	10.5	Nov. 7	7.1	3
5	Jan. 13	10.7	Jan. 18	5.9	5
6	Jan. 13	11.5			
7	Jan. 13	10.7	Jan. 18	5.9	5
8	Feb. 1	11.6	Feb. 15	5.7	14
9	Feb. 1	11.0	Feb. 10	6.5	9

dogs who were given magnesium immediately after operation showed symptoms of tetany on the fourth to twentieth day. Animal number 6 remained normal for ten days after operation but escaped from the laboratory before the completion of the experiment.

It has been demonstrated by Salvesen (1923) that milk contains enough calcium so that if fed in large quantities to parathyroidectomized dogs it will maintain the animals in a normal condition for long periods. Swingle and Wenner (1926) in experiments on the effects of strontium on parathyroidectomized dogs, have shown that dogs which develop tetany may be brought back to normal within a few days, by adding one pint of milk to the amount of strontium given per day. With magnesium administration alone it is difficult to bring about a recovery once tetany becomes manifest. The animals may be kept alive for some time by continuous magnesium treatment but the blood calcium remains below the tetany level, except

in the case of dog 2 where the disappearance of tetany following the administration of 300 cc. of a 5 per cent solution of magnesium lactate was accompanied by a rise in serum calcium from 6.1 mgm. to 8.97 mgm. per 100 cc. It was found that one-half pint of milk given along with magnesium daily for a few days will cause the abatement of tetany which is coincident with a rise in calcium level of the serum.

By this careful administration of magnesium for about forty days, parathyroidectomized dogs may permanently recover from tetany and, at the end of this time, may be placed on a full meat diet and the magnesium treatment discontinued. Thus dogs 2, 3, 7, 8 and 9 completely recovered and became adjusted to the loss of their parathyroids after periods of medication lasting 27, 28, 73, 56 and 34 days respectively. Animals 7 and 8 had many attacks of tetany during the periods of medication; in these cases the period was greatly lengthened.

Several interpretations have been ascribed to the readjustment that takes place in the body following the loss of the parathyroids. It has been suggested by Luckhardt and Rosenbloom (1922), Swingle and Wenner (1926) and others, that the recovery may be due to hypertrophy of accessory parathyroid tissue, or possibly to a compensatory action of other organs. However, this readjustment, as Inouye (1924) pointed out, is not a definitely fixed one and does not indicate the dispensability of the parathyroid glands. Two dogs reported by Inouye developed tetany after remaining free from tetany for long periods. Animals 7 and 9 reported here, developed tetany on the 27th and 36th day respectively after the magnesium administration had been discontinued and were on a full meat diet. It was noted that for some reason, probably lack of exercise, these dogs became constipated. Following the administration of enemata, tetany symptoms soon disappeared and never again recurred.

The importance of constipation in relation to tetany has been stressed by many investigators. Luckhardt and Rosenbloom (1922) observed that parathyroidectomized dogs may be kept alive indefinitely if given intravenous injections of Ringer's solution and if care is taken that the animals do not become constipated. Swingle and Wenner (1926) found that dogs kept free from tetany by strontium develop tetany very quickly if constipated. This has also been found true in experiments where calcium, lactose and galactose have been the therapeutic agents. It appears that the end products of intestinal putrefaction may exert an influence upon the predisposition of parathyroidectomized dogs to tetany, as Dragstedt maintained. All animals reported here were given enemata at various intervals during the experiment despite the fact that magnesium lactate acts to some extent as a laxative agent.

Magnesium acts in a way similar to calcium and strontium. As shown by Voegtlin and MacCallum (1911), the sedative effect on the nervous

system produced by magnesium is much greater than that produced by calcium or strontium. In dogs in tetany, it has been found here that oral administration of magnesium readily abolishes the extreme neuromuscular symptoms, and in some cases it renders the animal quite anesthetic. However, with the removal of the symptoms recovery does not follow the disappearance of tetany. An analysis of the blood reveals that the calcium is increased but to a small degree. If now calcium is supplied in amount just large enough to cause a rise in serum calcium, the magnesium tends to keep the serum calcium at this new level.

This fact would indicate that magnesium acts in another manner aside from its anesthetic action upon the nervous system. It is suggested that the magnesium, when in excess, takes care of the increased phosphates or in some way brings the calcium back into solution.

The result detailed here concerning the permanent recovery after forty days' treatment with magnesium, are not unique since there are several accounts in the literature of permanent recoveries brought about by treatment with various agents for a period of forty or more days. Luckhardt and Rosenbloom (1922) found that animals could be cured of all tetany symptoms following parathyroidectomy by continued intravenous injections of Ringer's solution. They state that Ringer's solution in which strontium had been substituted for calcium is almost as effective. After a period of four to five weeks of this treatment the dogs permanently recover and can be placed on a meat diet without ill effects. Dragstedt and Peacock (1923) kept dogs free from tetany indefinitely on a diet of lactose, bread and milk *ad libitum*. After six weeks their animals took a stock diet and remained normal for long periods. Luckhardt and Goldberg (1923) were able to keep dogs free from tetany by calcium therapy. Inouye (1924) found galactose as well as lactose efficacious in the prevention of tetany. Collip (1925) reported two dogs that were returned to the kennels as normal dogs after they had received daily injections of parathyroid extract for two or three weeks. Dragstedt (1925) and Swingle and Wenner (1926) found strontium an effective agent in the prevention of tetany. After forty days of strontium therapy dogs returned to normal, were placed on a full meat diet and the therapeutic agent discontinued.

The problem of why dogs permanently recover after a certain period of treatment with magnesium, is as yet unsolved. The same end is brought about by intravenous injections of Ringer's solution, administration of strontium, calcium and magnesium salts and diets containing large amounts of lactose and galactose, although the actions of the various substances administered are quite different. Whatever the actions of these substances may be, the ultimate readjustment that occurs in the organism after forty or so days of that specific treatment, must be the same.

It has been suggested that this adjustment is brought about by 1, the

hypertrophy of accessory parathyroid tissue, or 2, by other organs taking over the function of the parathyroid glands. Dragstedt, Phillips and Sudan (1923) observed that recently parathyroidectomized dogs are less resistant to toxins than dogs recovered for long periods. There is an increase in resistance or gradual return to equilibrium or normalcy. This gradual return has been noted here also where dogs at the end of the experiment required less magnesium than dogs recently operated upon. This gradual return could support either view. However, this final recovery does not indicate the dispensability of the parathyroid glands for, as pointed out before, any abnormal condition is capable of disturbing the equilibrium established in those "recovered" animals, with tetany resulting.

Recently in a series of experiments (Wenner, 1926) the writer has been able to keep parathyroidectomized dogs free from tetany by oral administration of ammonium chloride. After forty to sixty days of such treatment the ammonium chloride can be discontinued and the animals placed on a heavy meat diet without tetany resulting.

SUMMARY

1. Continuous oral administration of magnesium lactate is an effective agent in the prevention of tetany in parathyroidectomized animals.

2. Magnesium tends to keep the calcium content of the blood serum above the tetany level. It is suggested that magnesium, by uniting with the excess phosphorus, keeps the calcium in solution.

3. The sedative effect of magnesium greatly reduces the excitability of the central nervous system whereby the more violent symptoms of tetany are held in check.

4. Magnesium-treated thyroparathyroidectomized dogs, kept free from tetany for about forty days, become readjusted to the loss of the parathyroids and may permanently recover and may be placed on a full meat diet without ill effects.

5. The lactates of cadmium, sodium and potassium, tried in connection with the magnesium experiments, were found to be ineffective in preventing or relieving tetany.

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MORPHIN INTOXICATION IN ADRENALECTOMISED RATS

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In the first part of this paper sensitiveness of adrenalectomised rats to morphin will be discussed. The second part refers to changes in blood sugar observed in these conditions.

A. Albino rats survive indefinitely in a large proportion after adrenalectomy. Those that die do so within the first five days after operation. The surviving animals show a number of symptons for a time, amongst which is a definite hypersensitiveness to certain drugs. This fact has been demonstrated in rats by Boinet (1896, 1897). More recently Lewis (1920, 1921, 1923) published a methodical study of the question. The following drugs are more toxic for adrenalectomised rats than for normal animals of the same species: Neurin (Boinet, 1896); adrenalin (Schwarz, 1910; Kahn and Starkenstein, 1911; Lewis, 1921, 1923); atropin (Lewis, 1920); floridein (Schwarz, 1910); cobra venom (Lewis, 1920); cardiac venoms of oubangii (Boinet, 1896) or oubaio (Boinet, 1897); digitoxin (Lewis, 1920); curare (Lewis, 1920); papaverin and codein (Lewis, 1920); veratrin (Lewis, 1920); dead bacilli (Scott, 1923; Jaffe and Marine, 1924); diphtheria toxin (Lewis, 1920; Belding and Wyman, 1926; Molinelli, 1926); insulin (Lewis, 1923); potassium cyanide, nicotin, histamine and acetonitril (Crivellari, 1926). The action of convulsant drugs, such as strychnin and picrotoxin (Lewis, 1920) and tetanus toxin (Rogoff, 1926), is not modified. Adrenalectomised rats resist anoxemia as well as normal ones, according to Marti (1922).

This hypersensitiveness diminishes gradually and disappears as time passes. Lewis found this true for morphia (1921), Artundo for insulin (unpublished observations), Molinelli for tetanus toxin (1926) and Crivellari for potassium cyanide and nicotin (1926). To a certain extent this accompanies the disappearance of other symptoms such as sensitiveness to cold (Marval, 1926).

Stewart and Rogoff (1922) deny this hypersensitiveness to morphin and they are supported by Rogoff and Necker (1925). The references given by these authors show many important errors, so it will be necessary to give them in detail. Lewis (1920) observed that rats whose adrenals had been extirpated 8, 15 and 30 days previously died when injected with 0.001 mgm. morphin chlorhydrate per gram, the lethal dose for normal controls

being 0.4 to 0.5 mgm. per gram. Occasionally an operated animal did not present this hypersensitiveness. Rats whose left adrenal had been taken out two weeks before died with 0.1 mgm. per gram. Later (1921) Lewis found that increased sensitiveness to morphin diminishes or disappears gradually but follows no regular curve. A new series of unilateral adrenalectomies did not confirm previous results; sensitiveness was found normal.

Stewart and Rogoff (1922) maintained that "there is no foundation for the statement that adrenalectomised rats succumb to a very much smaller dose of morphin than normal rats." This definite conclusion was drawn from their experiments on three rats. One, operated 48 days previously and weighing 216 grams survived a dose of 0.043 mgm. per gram and died later with a dose of 0.32 mgm. per gram. A second one, 54 days after extirpation, survived a dose of 0.2 mgm. per gram and died later with 0.3 mgm. per gram. The third one succumbed to 0.44 mgm. per gram, 43 days after adrenalectomy. These results do not contradict those of Lewis; they confirm the conclusion of his second paper that as time passes hypersensitiveness disappears. Lewis (1921) observed that of two rats operated 45 days previously one survived a dose of 0.1 mgm. per gram and one succumbed to 0.5 mgm. per gram; one rat operated two months before died with 0.3 mgm. per gram; of two rats operated on six months before one died with 0.4 mgm. per gram and one survived 0.3 mgm. per gram; one rat operated seven months before died with 0.1 mgm. per gram. This second paper, published before Stewart and Rogoff's refutation, can by no means be considered a rectification of the first one, as Rogoff and Necker believe.

In 1923 Lewis gave more detailed results and noted that unilateral adrenalectomy, operative trauma of the adrenal region and splenectomy do not increase sensitiveness to this drug. Thyroidectomy also has no influence (Olds, 1910) or a very slight one (Busso, 1924). Scott (1923) fully confirmed Lewis' findings. Rogoff (1925) observed an increased sensitiveness to morphin in adrenalectomised rats. Six to ten days after operation animals may die with one-half or one-tenth of the lethal dose for normal animals. No significance was given to these results, as 85 per cent of the mortality occurs at that time. Rogoff and Necker (1925)[1]

[1] Unfortunately Rogoff and Necker quote Lewis' results with serious errors. They also attribute to him having operated *rabbits* and obtained a mean survival of 26 hours. *Dogs* were, in fact, used (Compt. Rend. Soc. Biol., 1921, lxxxv, 1214). A proof mistake—later corrected (Ibid., 1922, lxxxvi and lxxxviii)—that can be easily detected if the paper is read carefully and does not appear in the more extensive publication in Spanish (Rev. Assoc. Med. Argent., 1921, xxxiv, 1104), was the cause of some aspersion by Stewart (Physiol. Rev., 1924, p. 170). In those experiments both adrenals were taken out simultaneously and it was observed that when Richet's solution (chloral-morphin) was used as the anesthetic, death occurred in 12 to 15 hours. This was attributed to the anesthetic, as much longer survivals were obtained (as long as 60 hours) when chloralose was used.

maintain that "there is no evidence that any significant change in tolerance occurs."

To what causes can such discrepancy in opinions be attributed? The authors just mentioned obtain a definite survival of only 50 per cent in 312 rats, while Lewis in more than 400 rats has a mean of 60 to 80, and even 100 per cent in some series. Houssay has a survival of between 70 and 100 per cent. Marval in 63 rats followed during five weeks has only 17 per cent mortality with deaths occurring only in the first week. Lewis observed mortality occurring in the first 48 hours following operation and exceptionally on the third and fourth days. Scott says that when after the first week the animals are healthy in aspect, active, eating well and have not lost more than 10 per cent of body weight, survival is assured.

Rogoff and Necker have somewhat different results. In their series 99 deaths occurred, 27 in the first week, 47 in the second, 17 in the third and 8 in the fourth. This prolonged and irregular mortality may be attributed to bad conditions of the animals used. Lewis mentioned three principal disturbing factors: disease (e.g., chronic bronchopneumonia), exposure to cold (later confirmed by Marval) and defective feeding. Scott also insists on the importance of food. Houssay, Sordelli and Mazzocco and Lewis have made an interesting observation in this respect. Rats coming from a stock showing symptoms of bad nutrition (scanty fur, atrophy of the eye-ball, sluggishness) gave considerable mortality on adrenalectomy. Incidentally they were very resistant to insulin. Forty days after being fed with bread and milk, their aspect was normal and their mortality on adrenalectomy was considerably reduced. Molinelli has observed a similar fact in 33 rats operated on just after arrival in the laboratory: 19 of them died during the 14 days following the extirpation. At the same time 60 animals born and bred in the laboratory were operated and only 6 died in the first few days thereafter; the rest survived indefinitely. A fact that seems to indicate that Rogoff and Necker worked with rats in poor condition is the minimal lethal dose of morphin found by them,—0.25 to 0.4 mgm. per gram, whereas Lewis finds 0.4 to 0.5 mgm. per gram for normal animals.

Rogoff and Necker's experiments correctly read show also that there is an increased sensitiveness to morphin in adrenalectomised rats between the sixth and fifteenth day following operation. Table 1 summarizes their experiments. Table 2 shows (Rogoff and Necker's experiments), that this sensitiveness diminishes as time passes. They did not come to these conclusions because the irregular and prolonged mortality of their adrenalectomised rats obscured their results and made them believe that death was due to adrenal insufficiency perhaps increased by a powerful depressant.

Experiments. White rats were used. Both adrenals were extirpated under ether anesthesia following the technique described by Lewis. The

animals were kept in a room with uniform temperature; they were fed on bread and fresh milk, with a little yeast added once a week. Post-mortem control of the operation was always performed.

Results obtained are summarized in table 3. In 273 rats operated a mean survival of 55 per cent was obtained, the extreme figures being 47 and 63 per cent. Deaths occurred within the first five days following oper-

TABLE 1

Morphin intoxication in adrenalectomised rats

DOSE OF DRUG PER GRAM	INTERVAL AFTER ADRENALECTOMY	NUMBER OF RATS TESTED	NUMBER OF RATS SURVIVING	NUMBER OF RATS DEAD
mgm.	*days*			
0.04	10–11	5	1	4
0.05	7– 8	5	2	3
0.05–0.055	8–15	12	2	10
0.10	10–12	5	1	4
0.10–0.15	8–12	18	3	15
0.15–0.20	10–12	4	1	3

TABLE 2

Morphin intoxication in rats more than two weeks after adrenalectomy

DOSE OF DRUG PER GRAM	INTERVAL AFTER ADRENALECTOMY	NUMBER OF RATS TESTED	NUMBER OF RATS SURVIVING	NUMBER OF RATS DEAD
mgm.				
0.10–0.15	14–21	11	4	7
0.10–0.15	30–36	7	5	2

TABLE 3

Percentage of definite survival in white rats after double adrenalectomy

NUMBER OPERATED	SURVIVALS	NUMBER OPERATED	SURVIVALS
	per cent		*per cent*
20	55	25	56
26	58	28	54
19	47	30	63
29	55	26	58
30	53	40	48

ation. Of the 124 animals that died 62.1 per cent did so on the first day, 26.6 on the second, 8 on the third, 5.7 on the fourth and 0.8 on the fifth. None died later in this series.

Toxicity of morphin chlorhydrate for normal rats was determined (table 4). The lethal dose found was 0.4 mgm. per gram.

Adrenalectomised rats are considerably more sensitive; 0.04 mgm. per gram, one-tenth the lethal dose for normal animals, kills 69.2 per cent

two weeks after operation. Even five weeks after operation this hypersensitiveness persists, though not so marked (table 5). To avoid the possible effect of cooling, a series of 19 rats operated on two weeks previously was put in a thermostat at 30°C. immediately after injecting 0.04 mgm. per gram of morphin. Thirteen (68 per cent) died. The result is essentially the same as when this precaution was not taken.

B. Changes in blood sugar. It is a well-known fact that morphin produces hyperglycemia. We have confirmed this fact in rats but have observed, however, that it is true only for small doses. Large doses produce hypoglycemia.

TABLE 4

Toxicity of morphin chlorhydrate in normal rats

DOSE PER GRAM	SURVIVALS	NUMBER OF DEATHS	PER CENT OF DEATHS
mgm.			
0.04	9	0	0
0.1	13	2	13
0.2	8	4	33
0.3	10	5	33
0.4	2	10	83

TABLE 5

Toxicity of morphin chlorhydrate in adrenalectomised rats

DOSE PER GRAM	TIME AFTER OPERATION	SURVIVALS	NUMBER OF DEATHS	PER CENT OF DEATHS
mgm.	*weeks*			
0.01	2	16	36	69.2
0.04	5	27	3	10.0
0.08	5	5	10	67.0
0.1	5	7	8	53.4
0.2	5	3	16	81.3

Morphin chlorhydrate was injected subcutaneously in a number of rats; one-half, one, two and three hours later blood was taken from the heart (occasionally from the *vena cava*) and blood sugar determined by Folin and Wu's method.

In normal rats 0.04 mgm. per gram produces a marked hyperglycemia (as much as 0.2176 per cent) between one and two hours after injection (table 6). The glycemia of normal rats was found to be 0.09 per cent, extreme figures observed being 0.0725 and 0.1587 per cent; larger doses—0.3 mgm. and 0.4 mgm. per gram—produce hypoglycemia, sometimes as low as 0.0278 per cent (table 7).

Adrenalectomised rats injected with 0.04 mgm. per gram have hypoglycemia as do normal rats injected with 0.4 mgm. per gram (table 8). A

TABLE 6

Blood sugar of normal rats after injecting 0.04 mgm. per gram morphin chlorhydrate

WEIGHT	TIME AFTER INJECTION	GLYCEMIA
grams	*hours*	*per cent*
145	½	0.1111
155	½	0.1587
85	1	0.1586
115	1	0.2176
118	1	0.0685
98	1	0.1428
200	1	0.0875
275	1	0.1384
225	1	0.125
105	2	0.202
100	2	0.1666
90	3	0.0666
90	3	0.101

TABLE 7

Glycemia of normal rats after injecting large doses of morphin

DOSE PER GRAM	WEIGHT	TIME AFTER INJECTION	GLYCEMIA
mgm.	*grams*	*hours*	*per cent*
0.3	151	½	0.0689
0.3	118	½	0.0586
0.3	90	½	0.0488
0.3	126	1	0.0489
0.3	212	1	0.0305
0.3	96	1	0.0303
0.3	220	1	0.0344
0.3	230	1	0.0621
0.3	111	1	0.0454
0.3	128	2	0.0303
0.4	127	½	0.0666
0.4	112	½	0.0656
0.4	100	½	0.0432
0.4	120	½	0.0654
0.4	120	2	0.0326
0.4	168	2	0.0436
0.4	187	2	0.0657
0.4	156	2	0.0278
0.4	86	2	0.0842
0.4	99	3	0.0435
0.4	110	3	0.0367
0.4	204	3	0.0675
0.4	245	3	0.0634
0.4	256	3	0.0475

smaller dose, 0.01 mgm. per gram, produces hyperglycemia in adrenalectomised rats (table 9).

These experiments confirm the results obtained by observing the mortality after injecting morphin. Adrenalectomised rats behave, in their reaction to morphin, as more sensitive tests than normal animals.

TABLE 8

Glycemia of adrenalectomised rats injected with 0.04 mgm. per gram morphin

WEIGHT	TIME AFTER INJECTION	GLYCEMIA	OBSERVATIONS
grams	*hours*	*per cent*	
244	½	0.0576	Severe symptoms
256	½	0.046	Severe symptoms
201	1	0.079	Severe symptoms
185	1	0.066	Severe symptoms
120	1	0.045	Severe symptoms
170	1	0.1205	Slight symptoms
105	1	0.0655	Severe symptoms
80	1	0.044	Very severe symptoms

TABLE 9

Glycemia of adrenalectomised rats injected with 0.01 mgm. per gram morphin

WEIGHT	TIME AFTER INJECTION	GLYCEMIA
grams	*hours*	*per cent*
220	½	0.125
240	½	0.145
205	½	0.126
186	1	0.165
140	1	0.098
105	1	0.133
165	1	0.148
180	2	0.121
300	2	0.098
208	2	0.095
155	2	0.169
150	2	0.144
170	2	0.120
164	3	0.145
180	3	0.119
142	3	0.198

In order to see if the increase in bood sugar could be considered as a defensive reaction in so far as it might aid the organism in resisting the action of the drug, glucose was given to normal rats simultaneously with the injection of morphin. Doses of 0.05 gram glucose were injected subcutaneously one-half hour before giving 0.4 mgm. per gram morphin

chlorhydrate and every half-hour afterwards—in all 0.20 gram glucose. No protective effect was observed. Of 15 rats so treated 13 died and only 2 survived.

SUMMARY

1. Healthy rats, well fed and protected from cold, survive double adrenalectomy in a large percentage. Death, when it occurs, is seen within the first five days following operation.

2. Adrenalectomised rats, two weeks after operation, succumb in large numbers (69.2 per cent), to 0.04 mgm. per gram morphin chlorhydrate, a dose one-tenth of the lethal dose for normal rats.

3. Protection from cold, by keeping the injected animals at 30°C., does not alter these results.

4. Five weeks after adrenalectomy rats are still hypersensitive, though in a lesser degree; 0.08 mgm. per gram kills more than half the number injected.

5. Hyperglycemia is observed in normal rats injected with 0.04 mgm. per gram morphin chlorhydrate. Hypoglycemia is seen when 0.3 and 0.4 mgm. per gram are given.

6. Two weeks after adrenalectomy 0.04 mgm. per gram produces hypoglycemia in rats. Hyperglycemia in this condition is seen when only 0.01 mgm. per gram is injected.

7. Repeated glucose injections do not increase tolerance for morphin in normal rats.

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SENSITIVENESS OF ADRENALECTOMISED RATS TO CERTAIN TOXIC SUBSTANCES

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Boinet, in 1896 and 1897, first observed that after adrenalectomy grey rats are more sensitive to certain toxic substances. More recently Lewis (1920, 1921, 1923) confirmed this fact and made a methodical study on white rats, using a large number of drugs. Scott (1923, 1924), Molinelli (1926), Torino (1927) and Artundo (results unpublished) have also found an increase in sensitiveness in adrenalectomised rats.

The present paper deals with the same problem in respect to drugs whose action had not been determined by the authors mentioned: nicotine, acetonitril, ergamine (histamine phosphate). White rats were used. Both adrenals were taken out simultaneously under ether anesthesia following the usual technique. The animals were fed on bread and milk *ad libitum*. A slight loss of weight occurred in the days following the operation. The mortality ranged from 10 to 15 per cent (table 1). Together with the second series of ten rats, twenty-five operated on by Doctor Molinelli were observed in which no deaths occurred. The high mortality in the first series is explained by the fact that they were operated on during the winter and that care was not taken to avoid cold by maintaining a constant temperature in the room where they were kept. Several deaths occurred in normal animals at the same time. Afterwards this cause of error was eliminated and series 2 and 3 gave more satisfactory results.

The drugs were tested after at least fifteen days had elapsed since the operation. A post-mortem examination showed that extirpation was complete. A few controls were operated on without extirpating the adrenals; no mortality followed.

Potassium cyanide. Busso (1924) found that potassium cyanide is less toxic for thyroidectomised rats. Adrenalectomy produces the opposite effect. The drug was dissolved in an 0.1 per cent sodium chloride solution. The largest amount of fluid injected was 3 cc. so the concentration varied according to the quantity of drug given. This was done in all our experiments. Subcutaneous injections were always made. The following symptoms were observed immediately after the administration of the drug: the animals became restless, dyspnea appeared followed by convulsions and,

TABLE 1

Mortality after adrenalectomy

NUMBER OF RATS OPERATED	NUMBER OF DEATHS ON DAYS AFTER OPERATION									
	1	2	3	4	5	6	7	8	9	10
35		1	1	2			1	1	2	
10		1								
20			2		1		1			

TABLE 2

Potassium cyanide intoxication

DOSE OF DRUG	ADRENALECTOMISED RATS			NORMAL RATS	
	Weight	Days after operation	Results	Weight	Results
mgm. per kgm.	*grams*			*grams*	
5	130	15	Lives	130	Lives
6	120	18	Lives	130	Lives
6	125	18	Dies	125	Lives
7	165	18	Lives	150	Dies
7	195	18	Lives	185	Lives
8	200	18	Lives	180	Lives
8	145	16	Dies	135	Lives
8	200	19	Dies	170	Lives
8	140	23	Dies		
8	205	23	Dies		
9	125	21	Dies	135	Lives
9	170	21	Dies	140	Lives
9	135	21	Dies	140	Lives
9	150	23	Dies		
9	135	23	Dies		
10	110	20	Dies	95	Lives
10	130	20	Dies	120	Lives
10	185	20	Dies	155	Lives
10	160	18	Dies	195	Lives
11				140	Lives
11				145	Dies
11				140	Dies
11				210	Dies
12				130	Dies
12				135	Dies
12				145	Dies
12				160	Dies

Lethal dose for adrenalectomised rats 8 mgm. per kilogram.
Lethal dose for normal rats 11 mgm. per kilogram.

in some cases, death. The lethal dose for normal rats was 11 mgm. per kgm. It was 8 mgm. per kgm. for adrenalectomised rats (table 2). As time passes the hypersensitiveness diminishes. For rats operated on fifty-two days previously the lethal dose was 9 to 10 mgm. per kgm. Symptoms were less marked and death did not occur so suddenly (table 3).

Nicotine. The drug was dissolved in distilled water and injected subcutaneously. Symptoms occurred more promptly in operated animals. They were: dyspnea, erection of tail, violent convulsions followed by death. The lethal dose was 27.5 mgm. per kgm. for normal rats and 17.5 mgm. per kgm. for adrenalectomised rats 18 to 24 days after operation (table 4). Later—68 days after operation—this increased sensitiveness had disappeared (table 5).

Acetonitril. A watery solution of acetonitril (Merck) was given in subcutaneous injections. Death occurred four, five or more hours after-

TABLE 3

Potassium cyanide intoxication in adrenalectomised rats fifty-two days after operation

DOSE OF DRUG	WEIGHT	RESULTS
mgm. per kgm.	*grams*	
8	185	Lives
8	220	Lives
9	140	Lives
9	190	Dies
10	100	Dies
10	205	Dies
11	115	Dies
11	145	Dies

wards, very few and slight symptoms being present. The lethal dose for normal animals was 5 mgm. per gram. Hunt and Seidell (1909) found a lethal dose between 4 and 5 mgm. per gram. Adrenalectomised rats, 17 to 18 days after operation, are considerably more sensitive, the lethal dose being 0.05 mgm. per gram, one-hundredth of the lethal dose for normal animals (table 6).

Histamine. Ergamine (histamine phosphate) was injected in the *vena saphena* (never more than 1.5 cc. of solvent). Symptoms occurred almost immediately; frequently death, preceded by a short period of convulsions. At times death comes on gradually, preceded by intense depression and lachrymal secretion, the mucous membranes becoming very pale or else having a rosy tint.

The lethal dose for normal animals was between 0.5 and 0.7 gram per kgm.,—somewhat lower than that given by Voegtlin and Dyer (1924), 0.9 gram per kgm. Adrenalectomised rats were found to be considerably

TABLE 4

Nicotine intoxication

DOSE OF DRUG	ADRENALECTOMISED RATS			NORMAL RATS	
	Weight	Days after operation	Results	Weight	Results
mgm. per kgm.	*grams*			*grams*	
0.5	120	24	Lives		
1.0	125	24	Lives		
1.0	115	24	Lives		
2.0	145	24	Lives		
3.0	185	24	Lives		
4.0	170	24	Lives		
5.0	125	25	Lives		
5.0	160	25	Lives		
5.0	100	23	Dies	98	Lives
6.0	100	25	Lives		
7.5	140	25	Lives		
7.5	145	25	Lives		
10.0	130	25	Lives		
10.0	105	25	Lives		
10.0	143	26	Lives		
10.0	128	26	Dies	130	Lives
12.5	120	26	Lives		
12.5	167	26	Lives		
15.0	135	26	Lives		
15.0	142	18	Dies		
17.5	150	18	Dies		
17.5	160	18	Dies		
20.0	155	18	Dies		
20.0	175	20	Dies		
20.0	180	20	Dies	125	Lives
22.5				160	Lives
22.5				190	Lives
25.0	190	20	Dies	140	Lives
25.0				155	Lives
25.0				125	Dies
27.5				105	Lives
27.5				135	Lives
27.5				150	Dies
27.5				170	Dies
27.5				190	Dies
30.0				115	Dies
30.0				150	Dies
30.0				165	Dies
35.0				135	Dies
35.0				175	Dies

Lethal dose for adrenalectomised rats 17.5 mgm. per kilogram.
Lethal dose for normal rats 27.5 mgm. per kilogram.

more sensitive, the lethal dose being between 0.01 gram and 0.04 gram per kgm.

This fact has been found true for other species. Dale (1920) described

TABLE 5

Nicotine intoxication in adrenalectomised rats 68 days after operation

DOSE OF DRUG	WEIGHT	RESULTS
mgm. per kgm.	*grams*	
20.0	180	Lives
22.5	200	Lives
25.0	190	Lives
27.5	220	Lives
30.0	200	Dies

TABLE 6

Acetonitril intoxication

DOSE OF DRUG	ADRENALECTOMISED RATS			NORMAL RATS	
	Weight	Days after operation	Results	Weight	Results
mgm. per gram	*grams*			*grams*	
0.005	160	17	Lives		
0.01	135	17	Lives		
0.01	210	17	Lives		
0.05	128	17	Dies		
0.05	148	18	Dies		
0.05	227	19	Dies		
0.1	165	17	Dies		
0.1	168	18	Dies		
0.1	185	19	Dies		
1.0	145	16	Dies	150	Lives
3.0	165	16	Dies	165	Lives
3.0				150	Lives
4.0				155	Lives
4.0				180	Lives
4.5				170	Lives
4.5				160	Lives
5.0	125	16	Dies	135	Dies
5.0				144	Dies
5.0				156	Dies
5.0				170	Dies

Lethal dose for adrenalectomised rats 0.05 mgm. per gram.
Lethal dose for normal rats 5 mgm. per gram.

it in adrenalectomised cats; Kellaway and Cowell (1923) confirmed the finding and observed that cats with only the adrenal medulla extirpated were also more sensitive. Banting and Gairns (1926) maintain that the

lethal dose for adrenalectomised dogs is one-thirtieth that for normal animals. They believe that adrenal death is due to an intoxication produced by histamine or a histamine-like substance derived from protein. Symptoms are more severe in adrenalectomised animals, fall in blood pressure is greater as also blood concentration (Kellaway and Cowell).

Dale and Richards (1918) and Burn and Dale (1926) believe that there is an antagonistic action between epinephrin and histamine. In physiological conditions the equilibrium between these substances would regulate the capillaries and blood pressure. Histamine injection is followed,

TABLE 7

Ergamine intoxication

DOSE OF DRUG	ADRENALECTOMISED RATS			NORMAL RATS	
	Weight	Days after operation	Results	Weight	Results
gram per kgm.	*grams*			*grams*	
0.002	205	14	Dies		
0.005	169	14	Lives		
0.01	165	14	Dies		
0.02	210	14	Lives		
0.04	225	14	Dies		
0.05	215	14	Dies	190	Lives
0.1	150	14	Dies		
0.2	165	14	Dies		
0.2	195	14	Dies	185	Lives
0.3	170	14	Dies		
0.3	190	14	Dies	135	Lives
0.4				155	Lives
0.5				155	Lives
0.5				145	Dies
0.7				150	Dies
0.9				120	Dies
0.9				120	Dies

Lethal dose for adrenalectomised rats 0.01 to 0.04 gram per kilogram.
Lethal dose for normal rats 5 to 7 grams per kilogram.

according to Kellaway and Cowell, by an increase in adrenal secretion of short duration shown by the "paradoxical" dilatation of the denervated pupil in normal cats that is absent in animals with adrenals extirpated or the medulla destroyed by radium emanation.

Burn and Dale observe that small doses of histamine produce a drop in blood pressure followed by a rise. This rise they attribute to increased adrenal secretion, as it is missing when the adrenals have been previously extirpated, also because ergotamine inverts it. Small doses of epinephrin produce opposite effects,—a slight increase in blood pressure followed by a

fall, due probably to an increased output of histamine, as it is not seen when working on isolated organs. Lucas (1926) found a slight increase in the histamine content of the liver in adrenalectomised dogs.

Discussion. Our results are summarised in table 8. There is an increase in sensitiveness to the four drugs studied, specially marked for acetonitril (1:100) and histamine (1:12.5). The rats had completely recovered from the operation, their aspect was healthy and no spontaneous mortality was occurring when the drugs were tested. The control animals operated without taking out the adrenals gave no increase in sensitiveness.

Several theories can be advanced to explain the hypersensitiveness to drugs seen in adrenalectomised animals: 1. The toxic substances are not destroyed by the adrenal or by some mechanism that is disturbed by adrenal insufficiency. 2. The toxic action of the drug accumulates with the action of an endogenous poison, normally destroyed or transformed by the adrenal or some system that is disturbed by adrenal insufficiency.

TABLE 8

DRUG	ADRENALECTOMISED RATS		NORMAL RATS	RATIO
	Days after operation	Lethal dose	Lethal dose	
		mgm. per kgm.	*mgm. per kgm.*	
Potassium cyanide	16–23	8	11	1:1.37
Potassium cyanide	52	9		1:1.22
Nicotine	18–20	17.5	27 5	1:1.57
Nicotine	68	27.5		1:1
Acetonitril	16–19	50	5.000	1:100
Hystamine phosphate	14	40	500	1:12.5

3. General depression or depression of certain functions (which we cannot specify) owing to lack of a special substance secreted by the adrenal, or to a metabolic process disturbed by adrenal insufficiency. At this moment the toxic theory (existence of an endogenous poison) after being discredited for many years, is again gaining adherents, though nothing as yet permits definite conclusions.

SUMMARY

Two or three weeks after adrenalectomy albino rats are more sensitive to the toxic action of potassium cyanide (1:1.37), nicotine (1:1.57), acetonitril (1:100) and histamine (1:12.5).

This hypersensitiveness disappears for nicotine 68 days after the operation and is diminished for potassium cyanide 52 days after extirpation.

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THE REGULATION OF THE FLOW OF BILE

III. The Rôle of the Gall Bladder

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That the resistance to the flow of bile into the intestine lies in the tonicity of the duodenum and not in the activity of a separate sphincter at the mouth of the common duct has been demonstrated (Burget, 1925, 1926; Graham, 1926; Copher and Kodama, 1926). It has been suggested by Higgins and Mann (1926) and by Whitaker (1926) that filling of the gall bladder is made possible by the action of such a sphincter. This would seem to be ruled out on a priori grounds. If the resistance to the flow of bile lies in the tonicity of the duodenum this is the resistance by reason of which the gall bladder fills.

With the duodenum in a normal state of tonus the secretory pressure of the liver may bring about distention of the filled gall bladder. Thus pressure is exerted upon the contents. Besides this pressure produced because of the elasticity of the organ it has been claimed by some that there is active forceful contraction of its musculature when bile is needed in the intestine. If the gall bladder wall possesses such capabilities of contraction, it should, when in a balanced physiological condition, respond to one of the following possibilities of excitation: activity of the duodenum, as in digestion; a hormone that might be produced in the process of digestion; stretching, as takes place when it is distended with bile; drugs that effect stimulation through the nerve supply or that act directly on smooth muscle fibers.

Numerous investigations have been made to determine the contractile powers of the gall bladder and to establish the part it may play in the flow of bile. Among those who have presented experimental evidence in support of expulsion by forceful contraction are Boyden, Whitaker, Higgins and Mann, McMaster and Elman, Chiray and Pavel, and Mellanby. On the other hand, Graham, Copher and Kodama, Winkelstein and Aschner, Friedenwald, Martindale and Kearney, Wakerlin, Auster and Crohn, and Bainbridge and Dale have failed in their attempts to obtain evidence that would substantiate an active expulsion theory.

From an anatomical point of view the viscus is poorly supplied with

muscle tissue in comparison with the small intestine or the urinary bladder. Hendrickson (1898) made a careful study of its structure in man, dog and rabbit. Referring to the dog he makes the following statement: "The muscle bundles are not arranged in definite and regular coats; the transverse, longitudinal, and diagonal bundles are more or less separated from one another by a certain amount of connective tissue, but since the individual muscle bundles overlap, there are few if any places where muscle is entirely absent." He makes no separate description of the gall bladder in man but states, "what has been said of the gall bladder of the dog may be repeated of the gall bladder of man." Confirmation of this description was made by Sudler (1900). Speaking of the muscular coat of the gall bladder, Auster and Crohn (1922) state, "Sparsely distributed unstriped muscle fibers are interlaced with fibrous tissue in this layer."

These anatomical descriptions would not indicate strong contractile properties, yet in view of the wide differences of opinion held by investigators as regards its functions further study seemed necessary.

Experiments were carried out on twenty-six dogs and five cats. Under ether anesthesia the abdomen was opened in the midline and the gall bladder exposed. After carefully removing the bile, a small thin rubber balloon was inserted through an opening in the fundic region. This was held in place by a pursestring suture and connected by a small rubber tube with a water manometer of 9 to 10 mm. inside diameter. Care was taken not to produce torsion of the gall bladder or otherwise prevent normal position. In some experiments a balloon was also placed in the duodenum and similarly connected with a water manometer. Blood pressure was recorded from a cannula in a carotid artery. After the necessary surgery was completed the abdominal opening was covered with pads of cotton wrung from warm normal salt solution. The ether was usually removed at this time and barbital sodium given intravenously in quantity to maintain light anesthesia. The animal was covered and additional heat supplied by an electric light bulb and reflector.

Rhythmical tonus changes were observed in about 75 per cent of the dogs. They usually occurred at the rate of two or three per minute. This is similar to the rate found by Taylor and Wilson (1925), Wakerlin (1926) and Bainbridge and Dale (1905). That there seems to exist no direct relationship between these tonus changes and peristalsis of the duodenum is brought out in figure 1. The bile had been removed shortly before the tracing was made so that the gall bladder contained only the balloon with a pressure of 12 mm. water. The effect of eserin intravenously was quite marked on the intestine; however, gall bladder tonus was practically unaffected.

Some doubt has been expressed by Graham (1926) as to the active origin of such changes from the gall bladder. Their augmentation by barium

chloride, their disappearance under deep anesthesia, and the fact that they do not develop in edema of the viscus, seem to lend definite evidence of their physiological origin. Further evidence is brought out in figure 2. A rather unusual development of tonus rhythm rate took place. In the beginning every seventh or eighth wave was much stronger than the intervening ones. The stronger waves gradually appeared more frequently until at the end of 45 minutes normal rhythm had developed. Respiration was uniform throughout the period.

Pilocarpine, adrenalin and atropine have effects as indicated by the

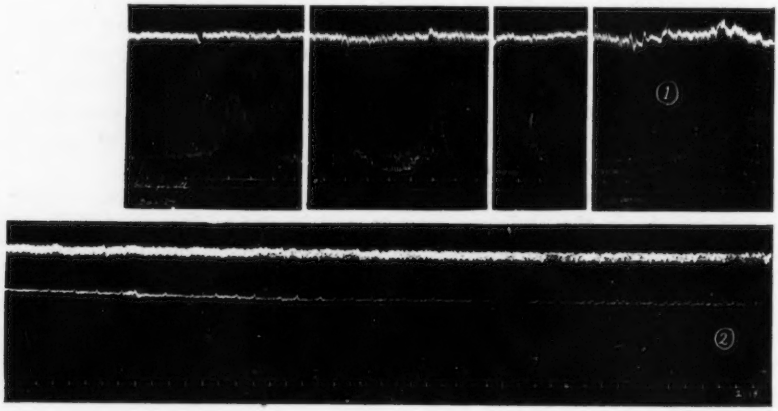

Fig. 1. Tracings from duodenum and gall bladder with blood pressure record. Time in minutes. Gall bladder tonus rhythm uninfluenced by tonus or peristalsis of duodenum. Eserin (1 mgm. intravenously) caused marked rise of tonus in duodenum but did not affect tonus of gall bladder.

Fig. 2. Unusual development of normal tonus rhythm. Blood pressure above and gall bladder tracing below. At the beginning a strong contraction occurred only after 6 or 7 weak contractions. Rate of the stronger gradually increased until after 45 minutes all had become of the stronger type.

innervation found by Bainbridge and Dale (1905) (figs. 3, 4, 5). Although the vascular changes in the liver were not eliminated, the effects of these drugs in most instances were not great. Eserin had an effect similar to pilocarpine. Definite augmentation of tonus rhythm followed intravenous injection of barium chloride. Vagus stimulation that caused contractions of the small intestine had little effect on the gall bladder. The results with drugs confirm the findings of Lieb and McWhorter (1915) on isolated strips of gall bladder wall.

Some difficulty was encountered by reason of edema developing in the gall bladder. No previous investigator has reported having met with

this phenomenon in similar studies, although Taylor and Wilson (1925) thought some breeds of dogs better than others and obtained rhythmical contractions in only 60 per cent of their dogs. The onset of the condition can usually be recognized within the first hour after insertion of the balloon. If within this time no tonus changes appear and the pressure begins to rise with respiratory oscillations becoming less pronounced, it is fairly certain that edema is developing. The progress is slow and a maximum is

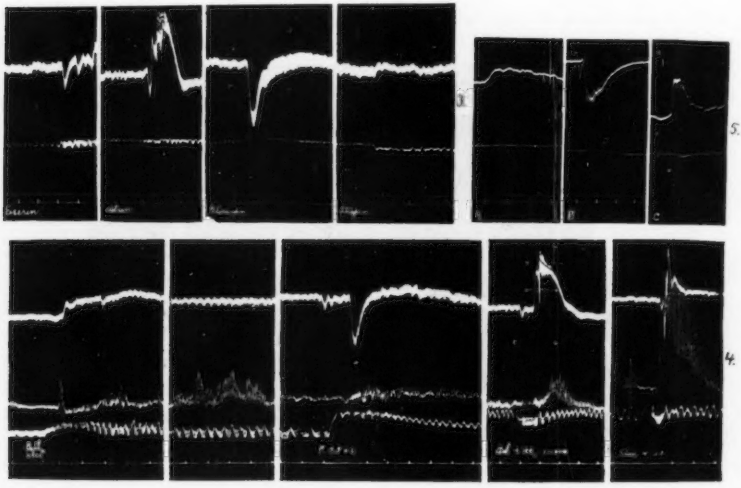

Fig. 3. Blood pressure above and gall bladder tracing below (25 mm. water pressure in balloon). Tonus change as affected by intravenous injection of 2 mgm. eserin, 3 cc. 1–50000 adrenalin, 2 mgm. pilocarpine, and 2 mgm. atropine.

Fig. 4. Middle tracing from duodenum, lower from gall bladder and upper, blood pressure. Showing effect of $BaCl_2$ (5 mgm.) on tonus and rhythmic contraction of gall bladder. Pilocarpine (0.5 mgm.) and adrenalin seem to affect tonus in opposite directions. Vagus stimulation affected gall bladder only slightly.

Fig. 5. Cat. Upper tracing blood pressure, lower gall bladder. At *A* barium chloride given intravenously; at *B*, pilocarpine; at *C*, adrenalin. Only slight effect seen by these drugs on gall bladder.

reached only after several hours. The wall of such gall bladders may be 5 to 6 times as thick as normal. Sometimes the edema was uniform throughout the organ and at other times confined more to one region than to another. This condition developed in approximately 25 per cent of the dogs even though care was taken to avoid large vessels in making an incision in the fundus and to prevent any bile coming in contact with the outer surface of the organ. Figure 6 shows the rate of development as judged by the rise in intra gall bladder pressure. The gradual increase

in pressure resembles that described by Higgins and Mann (1926) and characterized by them as a slow contraction.

In order to study the activity of the gall bladder during the digestion of a fat meal a slightly different technic was used. After the balloon was in place and the pressure within it brought to 15 to 20 mm. water, bile was reinjected into the gall bladder by a hypodermic needle pushed through the wall so as not to puncture the balloon and in an oblique manner in order that pressure would collapse the hole when the needle was removed. Thus the gall bladder was filled with bile and also contained a partially

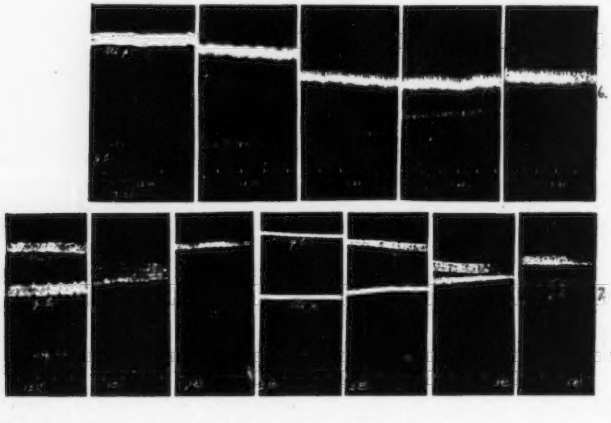

Fig. 6. The development of edema. Upper tracing, blood pressure; lower, gall bladder. Pressure in balloon increased from 15 mm. water to 85 mm. in two hours. Respiratory oscillations grew less as edema increased.

Fig. 7. Effect of fat meal on gall bladder. Preliminary to giving meal, balloon pressure in "empty" gall bladder set at 15 mm. water. Bile was injected in gall bladder causing pressure to rise to 85 mm. Fat meal given at 12:10. Pressure reached a maximum at 2:00 o'clock and fell rapidly until 3:00. At 5:30 pressure stood at 90 mm. and tonus rhythm was present. No contractions of gall bladder.

distended balloon. Should bile be expelled by muscular contraction, such contraction would be registered on the drum. On the other hand, if it merely showed increased pressure, due to secretion, until resistance at the duodenum was overcome, decreased pressure would follow with no signs of contraction. The secretion induced by the fat meal might be expected to cause an early rise in pressure since the gall bladder was already fairly well filled. Figure 7 illustrates an experiment of this nature. The balloon pressure in the "empty" gall bladder was 15 mm. This was raised to 85 mm. by injecting 8 cc. of bile. At 12:15 a meal of egg yolks and olive oil was given by stomach tube. The pressure soon began to rise and reached

a maximum of 140 mm. in one hour and 45 minutes. Tonus rhythm almost disappeared. The pressure fell rapidly for the next hour after which it fell more slowly. Five hours after the meal was given the pressure was 90 mm. and tonus rhythm was again pronounced.

It is obvious that there occurred no contraction of the gall bladder here unless it covered a period of a little less than two hours and relaxed in the same manner. This is impossible from our knowledge of smooth muscle contraction. Wakerlin (1926) obtained no contraction of the gall bladder following a fat meal. It is of interest to note here also that tonus changes can be no material factor because they disappear when most needed, that is, when the bile is under greatest pressure. We are forced to conclude that the rise in pressure was due to secretion of bile and that, when this pressure overcame the resistance at the intestine, bile began to flow. Tonus changes and peristalsis of the duodenum have been shown to cause this resistance to vary. A temporary relaxation of the intestine due to the fat meal is not apparent here probably because such relaxation was not enough to cause the resistance to fall below 85 mm. water.

At different times in the course of these experiments there has been some suggestion that as gall bladder pressure increases blood pressure falls. This may have been only a coincidence. Reflex inhibition of the heart and augmentation of respiration on manipulation and distention of the gall bladder have been observed. Contraction of the gall bladder was never observed to follow sudden distention.

Discussion. Whitaker (1926) and Boyden (1926), employing the x-ray after rendering the gall bladder opaque, studied the changes in the size of the organ in cats following a fat meal. They concluded that fats cause the gall bladdder to expel its contents in the course of a few hours by forceful contraction of its musculature. Although, following such procedure, there is a noticeable reduction in size, to deduce from this evidence that the gall bladder expels bile by forcibly contracting would seem unwarranted in view of its great elasticity. Man was found to give a similar response to a fat meal. Since the anatomy of this viscus in man is no different from that in the dog, it seems that the latter would be an ideal experimental animal for this study.

Boyden (1926), using the technic mentioned above, injected adrenalin intravenously into cats and claimed it to be "the most powerful excitant of the gall bladder yet found." This is contrary to the observations here presented and to the findings of Bainbridge and Dale (1905), Taylor and Wilson (1925), Lieb and McWhorter (1915), Mitchell and Stifel (1916) and Langley (1901). Adrenalin causes inhibition of the intestine and during this inhibition the resistance to the flow of bile has been shown to be reduced to a minimum (Burget 1925, 1926). It seems probable, therefore, that the results of Boyden and Whitaker can best be explained on the basis of relaxation of tonus in the duodenum.

Observing the exposed gall bladders of guinea pigs given a fat meal, Higgins and Mann (1926) found that there were changes in shape and reduction in size during the ensuing two and one-half hours. Activity of the duodenum was not considered. However, they conclude: "There could be no question but that the gall bladder was emptying through the cystic duct by its own muscular contraction." They further describe experiments on dogs whereby, under local anesthesia, the hepatic ducts were ligated and a small rubber catheter placed in the common duct and connected with a water manometer. A test meal was injected through a T-tube sutured into the duodenum. After 30 minutes to one hour the pressure gradually rose in the manometer reaching a maximum in three hours after the food had been given. They interpret this as lending further evidence that the gall bladder empties by its own intrinsic activity. Certainly this is not representative of the usual type of smooth muscle contraction and, in view of our observations on edema of the gall bladder, it seems entirely possible that such a process took place in Higgins and Mann's experiment from trauma and circulatory disturbance incurred in ligating the hepatic ducts and inserting a catheter in the common duct.

Using dogs that had undergone "triple intubation" several days previously, McMaster and Elman (1926) undertook to show that the gall bladder expels bile by forceful contractions. By means of a cannula in the neck of the gall bladder connected with a manometer of 3 mm. bore (a rise of 140 mm. would equal 1 cc.), they were able to study its activity in a healthy unanesthetized animal. While fasting the animal showed a gall bladder pressure of 100 mm. When fed, the pressure suddenly (time element apparently less than one minute) rose to 200 mm. returning to 115 mm. in about seven minutes. No further contractions took place until, twenty-five minutes after the first feeding, food was again given and the pressure rose to 250 mm. These are entirely different from the contractions in the dog as described by Higgins and Mann (1926) in that the contraction is of short duration and is precipitated immediately following taking of food and does not re-occur until food is again taken. Such contractions represent no greater volume change in the gall bladder than that brought about by rhythmical tonus changes in some of our animals. McMaster and Elman's animals were unanesthetized and were given food which Okada (1915) has shown increases the amplitude of the rhythmical contractions.

The claims of Chiray and Pavel (1926) and Mellanby (1926) that the gall bladder expels bile by muscular contractions are based upon observations made on the isolated strip and cannot be considered as lending strong evidence in support of the theory.

On the other hand Graham (1926) states, "Copher and Kodama have recently performed some crucial experiments, not yet published, which

can leave little doubt that whatever active muscular contraction there may be, it can at most play only a minor rôle in the emptying of the gall bladder. The chief factors in the emptying are purely passive and are explained on simple mechanical principles." Winkelstein and Aschner (1925) obtained no evidence of spontaneous contractions and concluded that drugs cause tonus changes only. Although watched for carefully in 34 dogs under various stages of anesthesia Friedenwald, Martindale and Kearney (1922) were never able to observe any actual contraction of the gall bladder.

Wakerlin (1926) carried out a number of experiments on dogs whereby a balloon was aseptically placed in the fundus of the gall bladder and held by a pursestring suture. After a few days the balloon was connected with a water manometer while the dog was quiet and under no anesthetic. Tonus changes were almost always present but by no means was he able to induce further contractions. Auster and Crohn (1922) were unable to invoke contractions with electric stimulation far stronger than that necessary to cause contraction of the small intestine.

The careful work of Bainbridge and Dale (1905) in studying the innervation of the gall bladder led them to believe that a part of the apparent change due to nerve stimulation and use of drugs was due to vascular change in the liver. Neither by direct nor by reflex stimulation were Winkelstein and Aschner (1925) able to obtain contractions suggestive that the organ might expel bile.

While these experiments were carried out under light anesthesia, it does not seem that this can be a severe criticism. Tonus changes were obtained that compare favorably with those reported by previous investigators without the use of an anesthetic. The fact that rhythmic tonus changes were present is sufficient evidence of the normal physiological state of the organ. If it would not respond under these conditions to the ordinary means of stimulating smooth muscle, a conclusion that it is incapable seems justified.

CONCLUSIONS

1. The rhythmical tonus changes occurring in the gall bladder are unaffected by peristalsis and tonus changes of the duodenum.

2. Drugs that act on the gall bladder by way of its nerve supply influence its tonus to a small degree. Barium chloride, acting directly upon the musculature, has a definite augmenting effect upon rhythmical tonus changes.

3. Following trauma or injury to its circulation the gall bladder may become very edemic. As edema develops tonus changes fail to appear and a gradually increasing pressure is exerted on its contents simulating the effect of a slowly developing contracture.

4. Because of its elasticity the gall bladder wall when under tension aids the flow of bile in a passive manner.

5. A fat meal does not cause the gall bladder to contract. However, the tonus of the small intestine is temporarily lowered by such a meal.

6. The theory of a reciprocal activity between the gall bladder and the sphincter of Oddi that brings about expulsion of bile seems to have no experimental basis.

7. The gall bladder is incapable of contractions that might be construed as being of major importance in the flow of bile.

8. Evidence that the gall bladder plays only a passive rôle in bile flow further confirms the conclusion that this process is regulated by tonicity and peristalsis of the duodenum, with elasticity of the gall bladder and intra-abdominal pressure as auxiliary factors.

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THE ACTION OF CHOLINE ON THE ALIMENTARY CANAL OF INTACT DOGS

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The essentially depressant action of choline hydrochloride on the tonus and motility of the empty stomach and on the tonus and motility of the digesting stomach in dogs not under anesthesia as reported by Mulinos[1] from our laboratory, would seem to question the universal applicability of the theory that choline is a peristaltic hormone of the alimentary tract. The literature on this theory is reviewed in the paper by Mulinos. It seemed desirable to extend Mulinos' observations on the stomach to the small and large intestines. The ideal technic for such observation would seem to be dogs prepared with permanent fistulae in three regions of the gut (the stomach, small intestine and large intestine), so that the motor activity of all three might be studied without anesthesia. But in the present study we used the less physiological method of barbital anesthesia, placing convenient sized rubber balloons through small openings in the stomach, various regions of the small intestines and in the transverse colon. This was done with the minimum of manipulation of the respective areas, the parts were immediately replaced in the abdomen and the abdomen closed, the temperature of the animal being kept up to normal. Prior to the experiments the animal was kept without food for 24 to 36 hours, so as to insure a relatively empty alimentary tract. The rubber balloons were connected with suitable water manometers and in which sufficient water pressure was introduced to register the tonus and contractions but not sufficient to overextend or injure the viscus. In some of the experiments simultaneous records of the blood pressure from the carotid artery were taken. The choline, dissolved in Ringer's solution, was slowly injected into the femoral vein.

RESULTS. The results are summarized in table 1, and illustrative tracings are reproduced in figures 1 to 3.

It will be noted that the action of choline hydrochloride on motor phenomena of the alimentary canal is quite variable. We may have pure inhibition of tonus and motility, we may have inhibition of tonus and mo-

[1] Mulinos: This Journal, 1926, lxxvii, 158.

TABLE 1

Action of choline chloride (intravenous) on the alimentary canal motility in dogs under barbital anesthesia. Balloon method

+ = increased tone or motility; − = decreased tone or motility; 0 = no motor effects.

EXPERIMENT NUMBER	CHOLINE	STOMACH	SMALL INTESTINE	LARGE INTESTINE
	mgm.			
1	10		− +	0
	20		− +	+
	30		− +	+
	40		−	+
	50		−	0
	50		− +	−
2	10	0	−	
	20	+	− +	
	30	− +	− +	
	30	+	− +	
	20	0	−	
	50	+	− +	
3	10	0	−	0
	10	0	−	0
	20	0	−	−
	25	+	−	+
	30	+	− +	−
	40	0	−	+
	50	−	− +	+
	40	+	− +	− +
	30	0	−	−
	20	0	−	−
	30	0	−	−
	40	+	− +	− +
	20	0	−	−
4	10	0	−	0
	20	+	− +	+
	30	0	− +	−
	40	+	− +	−
	Injected ½ mgm. atropine			
	40	+	− +	+
	30	+	− +	− +
	20	0	−	−
	10	0	−	−
5	30	− +	− +	
	40	+	− +	
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	20	−	−	
	50	− +	− +	

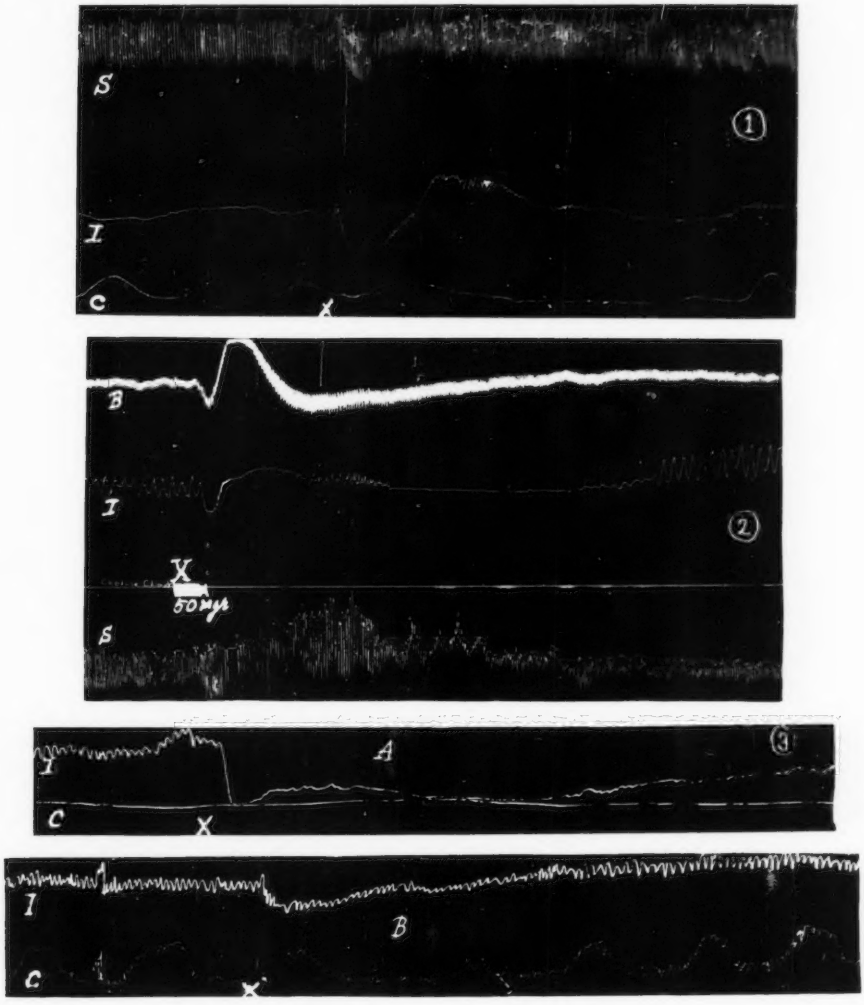

Fig. 1. Dog. Barbital anesthesia. Water manometer—balloon tracings. *S*, stomach; *I*, small intestine; *C*, colon. *X*, intravenous injection of 40 mgm. choline chloride. No effect on gastric tone and motility; inhibition, followed by increased tone in the small intestine; inhibition of motility in the colon.

Fig. 2. Dog. Barbital anesthesia. *B*, carotid blood pressure. Water manometer—balloon tracings. *S*, stomach; *I*, small intestines. *X*, intravenous injection of 50 mgm. choline chloride. Showing slight increase in gastric tonus, tonus inhibition followed by increased tone and depression of rhythmic contractions in small intestines.

Fig. 3. Dog. Barbital anesthesia. Water manometer—balloon tracings. *C*, colon; *I*, small intestines. *A*, *x*, intravenous injection of 50 mgm. choline chloride, showing prolonged inhibition of small intestines, no effect on quiescent colon. *B*, *x*, intravenous injection of 20 mgm. choline chloride, showing depression of tonus and motility both in colon and small intestine.

tility followed by increased tonus, or we may have increased tone and motility without any indication of inhibition. The latter result is rather the exception.

In all of our experiments the small intestine proved the most sensitive to the action of choline hydrochloride and the usual action on the small intestine was primary inhibition of tone and motility followed by increased tone. In many cases, however, the choline produced nothing but inhibition of tone and motility of the small intestine. This is particularly the case with the small doses of choline hydrochloride. The inhibition of tone and motility also predominates in the case of the large bowel but larger quantities of choline are required to influence this viscus. In a few cases, as indicated in table 1, choline produced a primary increase of tone in the transverse colon.

Under the conditions of our experiments the stomach is relatively insensitive to the action of this drug, but when choline is intravenously injected in sufficient quantities the usual effect is a temporary increase in the gastric tonus. This is contrary to the findings of Mulinos in the unanesthetized dog. In the unanesthetized dog Mulinos found invariably inhibitory effects produced by choline in the empty as well as in the digesting stomach, and inhibitory effects only. The explanation for this discrepancy is probably to be found in the difference in the tone of the stomach of Mulinos' dogs and in our dogs under anesthesia. When the stomach of a dog is in a state of hunger contractions the tonus is relatively high and when in a state of active digestive contractions there is also considerable tonus in the stomach. In barbitalized dogs, however, there is little or no spontaneous contraction in the stomach and consequently the viscus is relatively atonic. Our interpretation is that when the stomach has relatively high tonus the primary effect of choline is inhibition of the tone and motility. When the stomach is relatively atonic, choline in sufficient quantities to affect the motor mechanism, causes a slight increase in tonus.

Most of the past work on choline as a peristaltic hormone for the alimentary canal has been done on isolated parts of the gut, that is, they have been in vitro experiments. The physiological experiments of Mulinos and the present observations lend no support to the above theory, since we see that the nearer we approach the physiological condition of the alimentary canal the more variable the action of the drug on the motor mechanism the inhibitory action of the drug being in many cases as striking and as frequent as the motor action.

SUMMARY

In dogs under barbital anesthesia choline chloride (intravenously) produces the following motor effects on the relatively empty alimentary canal.

1. Stomach, usually a slight and temporary increase in tone by large doses of the drug.

2. Small intestine, usually a temporary inhibition of tone and motility followed by a temporary increase of tone, but there may also be pure inhibition of tone and motility on pure augmentation of tone.

3. Large intestine, usually a depression of tone and motility, and occasionally a slight increase in tone.

4. Under the conditions of our experiments the stomach and the large intestine are less sensitive to choline than the small intestine.

5. It seems difficult to interpret the above results, as well as those of Mulinos, on the theory that choline is the normal stimulus to gut motility.

STUDIES ON THE HEMODYNAMIC ACTION OF SUBCUTANEOUSLY INJECTED EPINEPHRIN

I. Some Conditions Under Which Hypodermically Administered Epinephrin Gives A Pressor Effect

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The writers undertook the systematic investigation of a problem in the course of which it was necessary to inject various amounts of epinephrin subcutaneously. If the animal (dogs were used in these experiments) happened to be under light anesthesia, a slight pressor effect was noted occasionally. This casual observation led us to investigate the question of the alleged possible dissociation of epinephrin action. In the literature we usually find the assumption of such a dissociation, namely, that the subcutaneously injected epinephrin produces a glycogenolytic, and the intravenously injected drug a hemodynamic effect only. The study of the literature convinced us that such an investigation is necessary because of the conflicting results on the possible pressor action of the hypodermically injected epinephrin.

Literature. As early as 1898, not long after the hemodynamic effect of the intravenously injected suprarenal extract had been discovered, we find S. Vincent (1898) investigating the effect of the subcutaneously injected drug on blood pressure. He could not detect any effect of the subcutaneously injected epinephrin, stating: "I have ascertained that in the cat, at any rate, doses sufficient to kill do not raise the blood pressure within half an hour of injection beneath the skin."

S. Amberg (1903), experimenting on dogs, injected huge amounts (as much as 5 to 53 mgm. per kilo body weight) of epinephrin beneath the skin and obtained in two cases definite pressor effects. His results are, however, open to criticism, not only because of the insufficient number of dogs used in these experiments, but mainly because he injected 37 and 16 mgm. epinephrin per kilo, doses sufficient to kill both animals $2\frac{1}{2}$ hours after the injection. Besides in one of his animals the maximal pressor effect was synchronous with the formation of a clot in the cannula, an event which may alone explain the rise of mercury.

T. R. Elliott (1905), like his predecessors, also injected fatal doses of

epinephrin subcutaneously and noticed only occasionally a slight elevation of blood pressure never exceeding 20 mm. of mercury. The difference between the hemodynamic effect of the intravenously or subcutaneously injected epinephrin he explains on the basis of slow absorption of the drug in the latter case.

Boruttau (1899) flatly denies any possible elevation of blood pressure after the hypodermic injection of the drug on the basis of his experiments on cats.

Patta (1905) attempted to give an adequate explanation of these conflicting results. Like Boruttau and Vincent he never obtained any pressor effect after the subcutaneous injection of epinephrin, unless he injected the drug perchance into a small venule. He assumes therefore that the authors reporting positive results injected the drug intravenously by piercing a small subcutaneous vein, rather than subcutaneously. Patta extracted the skin and subcutaneous tissues two hours after the injection of epinephrin and injected this extract intravenously. The marked elevation of blood pressure following this injection convinced him that epinephrin is not destroyed beneath the skin, at least within two hours. It is quite interesting that the results of Patta were disregarded in the later literature.

Meltzer and Auer (1905) investigated the hemodynamic action of the intramuscularly and subcutaneously injected epinephrin. They found that the intramuscularly injected drug acts promptly. The type of response resembles very much the effect following intravenous injection. The subcutaneous injection of the drug gave only feeble pressor effects (20 mm. Hg) and the rise and fall of pressure were not abrupt as in the case of the intravenous and intramuscular injections, but slow and extended like pituitrin. In one case a published tracing records massage of the injected area, but they never mentioned this procedure in the description of their methods or results, or in the subsequent discussion. It is not clear, however, that even in this one experiment the slight and slow elevation of pressure, obtained by Meltzer and Auer, is due to the injection of the drug or merely to the usual recovery of blood pressure following the change in the depth of anesthesia.

Janeway (1907), in a clinical study of blood pressure, published a curve demonstrating the absence of pressor effect after the subcutaneous injection of epinephrin in man.

Eppinger and Hess (1909) state that in some diseases (Graves' disease) there is an increased epinephrin sensitiveness. They paid no attention to the blood pressure changes, but noticed only an increased pulse rate and glycosuria after subcutaneous administration of the drug.

In 1911 the Dutch investigator, E. van Leersum, corroborated again those workers who failed to obtain blood pressure elevation after sub-

cutaneous injection of epinephrin. He could not detect hemodynamic effects following the hypodermic injection of 1 mgm. of epinephrin in the rabbit.

Starkenstein (1911) was at first inclined to admit the dissociation of epinephrin and considered even the possibility that different nerve-endings are stimulated by the intravenous and by the hypodermic injections. He reports *one* experiment, however, which convinced him that such an assumption is unnecessary. He injected 0.003 gram of epinephrin beneath the skin of a rabbit and states that he obtained a rise in pressure of 72 mm. Hg. But his figures show that his statement is incorrect, since the maximal rise obtained in this one experiment did not exceed 32 mm. of mercury.

The evidence in favor of absence of hemodynamic effects after hypodermic injection of epinephrin was thus slowly accumulating; and in his monograph on internal secretions Biedl (1913) gave an expression to the general belief when he denied the hemodynamic effect of the subcutaneously injected epinephrin.

From then on (1913) we find no data concerning the study of blood pressure after subcutaneous administration of epinephrin in animal experiments. The subsequent work, which we will discuss briefly, was carried out by clinicians, working on human subjects. Thiess (1914) observed slight pressor effects (18 to 25 mm. of mercury) following subcutaneous injection of the drug. Since the hypodermic injection of epinephrin was introduced in the practice of medicine either as a therapeutic measure (bronchial asthma) or as having diagnostic value, we can understand easily that clinicians became interested in the subject.

E. Goetsch (1918–20) is credited as having introduced epinephrin as a diagnostic agent. He found that exophthalmic goitre patients are usually more sensitive to subcutaneously injected epinephrin than normal individuals and react to the drug with (marked) tachycardia, decrease in the rate of respiration, and some elevation (around 30 mm. Hg) of blood pressure and an increase in the clinical manifestation of the disease.

Clough (1920) injected 1 cc. (1 mgm.) subcutaneously and noticed, like Thiess and Goetsch, a definite rise of the blood pressure. He could not confirm Goetsch, so far as the special epinephrin sensitiveness of exophthalmic goitre patients is concerned, but attempt to show that different individuals, irrespective of the condition of their thyroid, show a variable response to epinephrin responding with a blood pressure elevation from 20 to 100 mm. of mercury following the subcutaneous injection of 1 mgm. of epinephrin.

Fornet (1922) was also in position to notice slight pressor effects and tachycardia after the subcutaneous administration of the drug and showed that epinephrin, when injected hypodermically, is not destroyed at least for 20 minutes. He apparently was not familiar with the work by Patta.

Smith (1919) used the Goetsch test as a diagnostic agent, but only in connection with other tests. He does not question the reliability of the test, but makes the statement that the theoretical basis of it is not entirely sound. The Goetsch test is based essentially on the assumption of Cannon and his associates that in hyperthyroidism the sympathetic system is unusually sensitive, and epinephrin, being par excellence a sympathetic stimulant, should act especially vigorously in that condition. Smith maintains, however, that there are a number of conditions in which we may find an increased sympathetic sensitiveness.

Lyon (1923) published two extensive contributions on this subject. In his study on the absorption of epinephrin in man he states that the drug when given hypodermically is usually rapidly absorbed, probably by lymphatic channels and the speed of this process may be influenced by the circulation rate. In the majority of cases the pressor effect commenced within two minutes after the hypodermic injection and the maximum elevation occurred 6 to 40 minutes after the injection. He concludes that "the response to adrenalin bears a logarithmic relationship to the dose employed."

In another study Lyon gives a detailed account of his researches of various reactions to adrenalin in man following hypodermic injections. He describes the appearance of a small pale area just proximal to the needle mark and this blanched area increases in size for an hour or so and may still be visible for 3 or 4 hours later. Among the phenomena which follow the absorption of that drug, he emphasizes palpitation, tremor of the fingers, deep and often irregular breathing, and rise of the systolic blood pressure. The amount of increase ranged from 5 to 65 mm. Hg. Like Clough, the author believes that the type of reaction depends to a great extent on the "sensitiveness" of the patient and admits a wide individual variation. He states, however, that untreated hypothyroid cases show poor reactions (in 6 cases a rise of from 5 to 15), and hyperthyroid cases (11 subjects, a rise of from 20 to 44 mm. Hg) respond markedly to epinephrin.

Foerster and Benkovics (1926) obtained only slight pressor effects and tachycardia after subcutaneous administration of epinephrin. These investigators merely confirmed Fornet's results.

Heilig and Hoff (1926) published very remarkable results. According to them the subcutaneous injection of 1 mgm. of epinephrin is followed by an appreciable rise of the systolic blood pressure (10 to 35 mm. Hg), but only when the patient is in the *wakeful* state. During *sleep*, however, the hypodermic injection of the same amount of epinephrin did not elicit any definite pressor effects.

The study of the literature just reviewed shows that there is no conclusive evidence for unquestionable hemodynamic effects after hypodermic injection of epinephrin in animal experiments. In human subjects most

clinicians described comparatively slight pressor effects. The work of Heilig and Hoff, however, warrants caution in interpreting the clinical results. We know very well that fear and nervousness, which usually accompany any injection, and even taking the blood pressure, may well account for some blood pressure elevation. Such psychic effects are obviously excluded in sleep and indeed, according to Heilig and Hoff, one obtains hemodynamic effects after the hypodermic injection of epinephrin only in the wakeful state.

GENERAL EXPERIMENTAL METHODS. 1. *Anesthetic and analgesic.* Small dogs were used throughout (6 to 10 kgm.). In most of the experiments the animals were anesthetized by the minimal anesthetic dose of paraldehyde given orally, namely, 1.5 cc. per kgm. body weight. If this dosage was insufficient for the further preparation of the animal, ether was administered solely for the purpose of inserting a tracheal cannula and a 3-way cannula into the left carotid artery. As soon as these operations were completed etherization was discontinued and the animal subsequently kept quiet (if necessary) by means of $\frac{1}{4}$-1 grain of morphine sulphate subcutaneously injected.

A few experiments were performed under heavy morphine analgesia reinforced by ether solely for the insertion of tracheal and carotid cannulae. Since a few experiments performed under barbital sodium anesthesia (225 mgm. per kgm. intravenously injected) yielded negative results we continued our work under paraldehyde anesthesia as described above.

2. *Adrenalin injection.* Subcutaneous injections of adrenalin-HCl were now made singly or successively in various parts of the body. Originally we made them most commonly in the right and left lower quadrants of the anterior abdominal wall. This site is usually quite avascular from the point of view of macroscopic examination. From the point of view of subsequent massage, it is undesirable; for the massage is likely to involve the viscera underlying the abdominal wall and, furthermore, lacks the base against which a localized and more or less vigorous massage can be performed. We therefore chose the thorax, injecting the adrenalin in, or to the right and left of, the median line. Some injections were also made in the subcutaneous tissue of the extremities and a few in the head.

3. *Massage.* We would not have the reader infer from what has just been said that vigorous massage was essential to the success of the experiment. The latter is dependent on a number of conditions which will be described presently. Suffice it to say that the injected area was rubbed more or less gently with the index and middle fingers for some 20 seconds. In particularly sensitive preparations even a light stroke of the finger over an injected area sufficed to give an unmistakable pressor effect. Neighboring areas massaged as such or after being injected with equal amounts of H_2O or saline solutions served to control the results obtained from the "adrenalized areas."

4. *Dosage.* The dosage of adrenalin employed amounted, on the average, to 0.54 cc. of 1/1000 adrenalin hydrochloride solution per kilo body weight (i.e., 4.1 cc. 1/1000 adrenalin HCl to 7.6 kgm. dog). Extremes: 4 cc. to a 5 kgm. dog; 3 cc., 0.32 cc. per kgm. to a 9.5 kgm. dog.

5. *Miscellaneous.* Variations in the above technique for purpose of establishing the fact that we were dealing with a definite adrenalin effect and for studying the conditions which influence the response favorably or unfavorably will be introduced in a presentation of the data.

The base line in all our tracings represents 0 mm. Hg pressure.

RESULTS. *1. On the general results from massage of an adrenalinized area.* Massage of the injected area is a condition "sine qua non" for the successful outcome of the experiment. As just indicated, such massage need not be vigorous at all. If a pressor response appears without massage, the adrenalin, in our opinion, has gained direct access to a venule.

As might be expected, the pressor response to massage does not appear immediately. On the basis of numerous observations we have found it to begin about 17 seconds after massage of the area was commenced.

Neither is a response to be expected immediately after the injection of an area even if the dosage is as high or higher than that reported by us. The hemodynamic effect appears gradually, usually increases with time and after reaching a fastigium assumes the same height on repeated massage for a surprisingly long time. Figure 1 gives an instance of the development of the reaction. Four minutes, *B*, after the injection, massage gave only a doubtful response. Seven minutes later the pressor effect is unmistakable after 30 seconds or more of stimulation. Six minutes later the elevation of blood pressure following massage is striking, *E*. A much shorter period of massage, *F*, forty minutes after the injection gives not only a marked elevation of blood pressure but central cardiac inhibition seen so often after the intravenous injection of adrenalin.

The general character of the pressor effect is ordinarily identical with the response observed after the intravenous injection of epinephrin. However abrupt the rise in pressure might be in a given instance the return of the blood pressure to normal is usually slightly more protracted or even lengthened out so that the curve obtained may strongly simulate one seen after the intravenous injection of pituitrin. (See fig. 6 and fig. 7, *b*.)

Whereas we usually massaged an adrenalinized area for 20 seconds to obtain an effect, a one-second massage sufficed sometimes when we were dealing with a particularly sensitive preparation (see figs. 3 and 4).

The latter tracing illustrates the suspension of respiration commonly observed during the development of the pressor effect and its persistence while the blood pressure reaction was at its height. It is seen in the tracing as a suspension of the Traube-Hering waves.

The magnitude of the response as measured in terms of a rise in milli-

meter of mercury is quite variable. A cursory examination of the published tracings shows that the response, once developed in a given animal, may vary between 50 and 200 mm. Hg (fig. 8). The response depends in part, but not entirely so, on the amount of adrenalin subcutaneously injected. A number of conditions, some of which will be discussed presently, must be present to obtain any response whatsoever. Before discussing these conditions we wish to cite in support of our contention that the pressor effects observed were due in all cases to adrenalin and not to reflex vasomotor effects due to stimulation of the nerve endings in the subcutaneous areas made hyper-irritable by the irritant action of adrenalin, for it was suggested early in this investigation that the moderate responses seen at that time might be due to such a mechanism.

1. The mere magnitude of the response with all the qualitative earmarks of adrenalin action (abrupt rise, evanescent character, central cardio-inhibitory effect) would strongly suggest the probability that the result was due directly to massage of adrenalin into the circulation.

2. The fact that massage of a control area as such or after injection of water or saline solutions invariably given under the conditions of the experiment either no effect or a depressor effect under light anesthesia lends further support to the view (fig. 1, *c* and fig. 5, *b*).

In fact, spontaneous movements of the animal because of the lightness of anesthesia or movements elicited as a result of stimulation of the animal during the act of massage always gave a *prompt* depressor effect with a slow return of the blood pressure to normal (fig. 5, *a* and *c*).

3. The pressor effect furthermore first became evident 17 seconds after the beginning of massage—a delay longer than might be expected in a simple vasomotor reflex of a pressor character.

4. The intravenous injection of moderate amounts of cocaine HCl usually intensifies the pressor effect obtained from massage of an adrenalized area (fig. 6).

5. Ergotamine tartrate injected intravenously in suitable doses converts the usual pressor effect into a depressor effect (fig. 2).

II. Conditions affecting the appearance of the response and its magnitude. 1. *Time of injection.* It was pointed out that a pressor effect was not elicited immediately after the injection of adrenalin but that the response develops in time quite independent of the amount of the drug injected. This we related to the intense primary vasoconstriction preventing the absorption even with aid of massage because of complete or almost complete cessation of circulation in the area injected. The intense blanching of the skin overlying the area supports this explanation. Still more pertinent is the fact that injecting an area with $NaNO_2$ prior to the injection of adrenalin in the same area prevents certainly the maximum vasoconstrictor action of the adrenalin. As a result, the pressor effect can be

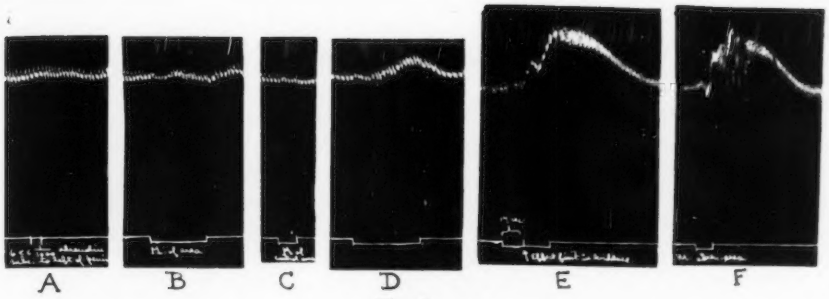


Fig. 1

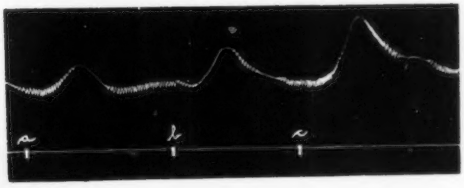


Fig. 3

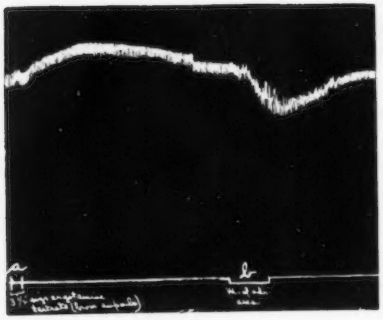


Fig 2

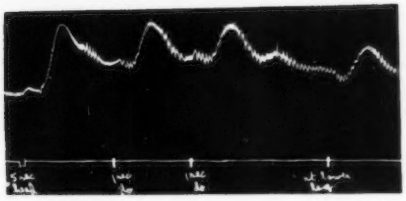


Fig. 4

Fig. 1. Blood pressure record showing the gradual development of the pressor effect on massage of a subcutaneous area previously injected with adrenalin hydrochloride. *A* = Subcutaneous injection of 6 cc. 1/1000 adrenalin-HCl in the hypogastric region. *B* = Massage of this area 4 minutes later. *C* = Massage of a neighboring skin area (control area). *D* = Massage of the area injected in *A* 11 minutes after the injection and 4 minutes after massage of the control area. *E* = Massage of area injected in *A* 17 to 18 minutes after the injection. *F* = Massage of the same area 40 minutes after the injection. In addition, tracing *E* shows the usual time of onset of the pressor effect (17 seconds) from the beginning of massage; and tracing *F*, more clearly than *E*, shows a typical central vagus inhibitory effect as seen commonly after the intravenous injection of adrenalin.

Fig. 2. Blood pressure record showing the reversal of the usual pressor effect following massage of an adrenalinized subcutaneous area, *b*, as the result of the intravenous injection at *a* of 3½ mgm. ergotamine tartrate.

Fig. 3. Blood pressure record showing effects of *one* second massage of 3 adrenalinized subcutaneous areas each containing 4 cc. 1/1000 adrenalin-HCl solution. These responses were 3 of a total of 24 obtained from these areas in the course of the experiment (weight of dog, 6 kgm.; 8 cc. paraldehyde per os + ⅛ gr. morphine sulphate subcutaneously).

Fig. 4. Pressor effects of a striking character obtained as the result of the massage of adrenalinized areas for as little as one second. These responses were obtained from a particularly sensitive preparation a long time after the subcutaneous injection of 4 cc. 1/1000 adrenalin-HCl. This tracing shows particularly well the suspension of respiration commonly seen at the height of the pressor effect resulting from the massage of an adrenalinized area.

obtained on massage within a few minutes after injection (fig. 7, *b*). The same results were obtained by causing the animal to inhale amyl nitrite At the height of the nitrite effect, adrenalin was injected subcutaneously. Massage of the area *immediately* after injection gave a pressor response. We are of the opinion that the pressor effect begins when the intense local vasoconstriction gives way to a secondary vasodilatation. As a matter of fact, such injected areas often appear pink at the time pressor effects can be elicited from them by massage. How soon the vessels from the injected area will escape from control of the adrenalin because of a secondary paretic effect cannot be foretold.

2. *The type and depth of the anesthetic used.* The depth of anesthetic affects markedly the results obtained. Because of its mild action paraldehyde was chosen, reinforced with morphine. We have on more than one occasion failed even under paraldehyde when, using the usual minimal dose, the anesthesia was particularly profound because of an individual susceptibility of the animal. We never succeeded under barbital sodium anesthesia but are not prepared to state that it will never be obtained

Fig. 5. Blood pressure tracing showing the depression of blood pressure as result of spontaneous movements of a lightly anesthetized animal, *a;* the absence of any effect if the movements of the limbs are only moderate, *b:* and a pressor effect neutralizing the depression in blood pressure as result of the massage of an adrenalinized area, *c*, which initially caused a depressor effect because of movements of the animal.

Fig. 6. Blood pressure records showing the synergistic action of cocaine-HCl on the pressor effect following the massage of a subcutaneous area injected with 6 cc. 1/1000 adrenalin-HCl some 3½ hours previously. (Weight of dog, 9.5 kgm.; 2 cc. paraldehyde per kgm.) *A*, *a*, Massage of a control skin area. *A*, *b*, Massage of the subcutaneous area injected 3½ hours previously with 6 cc. 1/1000 adrenalin-HCl solution. *B*, *c*, Intravenous injection of 3 cc. 0.3 per cent cocaine-HCl solution. *B*, *d*, Massage of the adrenalinized area, *A*, *b*, showing not only an earlier appearance of the pressor effect and a greater effect, but also a striking central cardio-inhibitory response which probably effectively reduced the pressor effect.

Fig. 7. Blood pressure record showing both the usual negative effect on immediate massage of an adrenalinized area and the almost immediate pressor effect obtained if the area chosen for the adrenalin injection is previously injected with sodium nitrite. *A* = Subcutaneous injection of 4 cc. 1/1000 adrenalin in a 5 kgm. dog under paraldehyde anesthesia (1.4 cc. per kgm.). *M* of *A'* = Massage of this area. *B* = Subcutaneous injection of 20 mgm. of sodium nitrite. *a* = Massage of area injected with sodium nitrite, *B; B'* = Subcutaneous injection of *B* with 4 cc. 1/1000 adrenalin-HCl. *b* = Massage of the subcutaneous area containing both sodium nitrite (20 mgm.) and adrenalin (4 cc. 1/1000).

Fig. 8. Blood pressure record showing a pressor effect of 160 to 200 mm. Hg as the result of successive massage of two areas, injected some time previously, each with a total of 4 cc. 1/1000 adrenalin hydrochloride solution. The rise in blood pressure as result of massage of the 2nd area, *B*, superimposed upon the rise resulting from massage of the first area, *A*, was so great that it was found necessary to clamp off the tube leading to the manometer (at *a*) in order to prevent emptying of the manometer of its mercury.

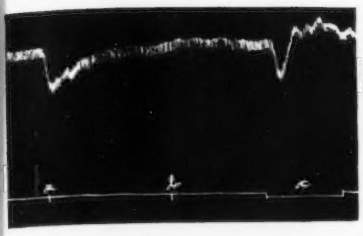
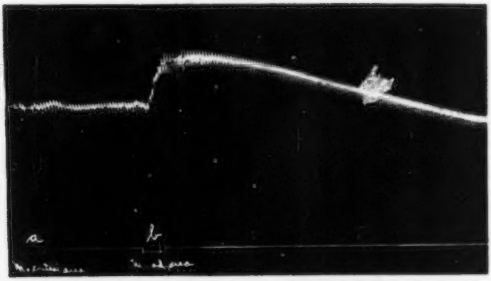


Fig. 5



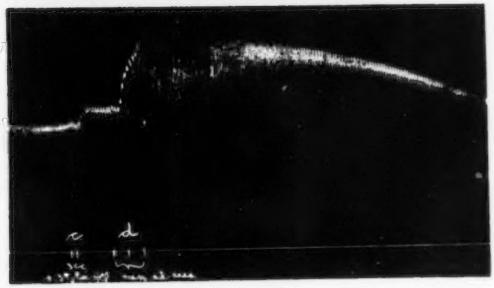


Fig. 6

Fig. 8

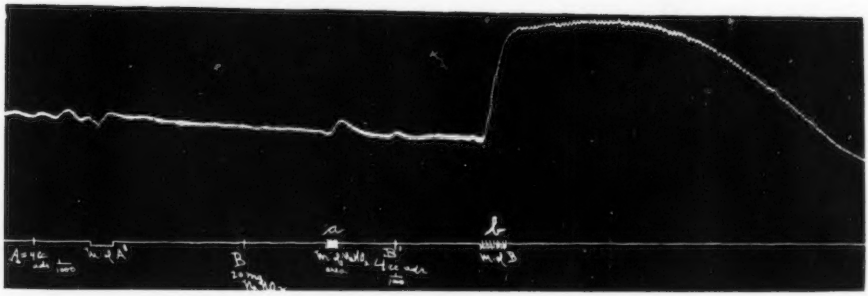


Fig. 7

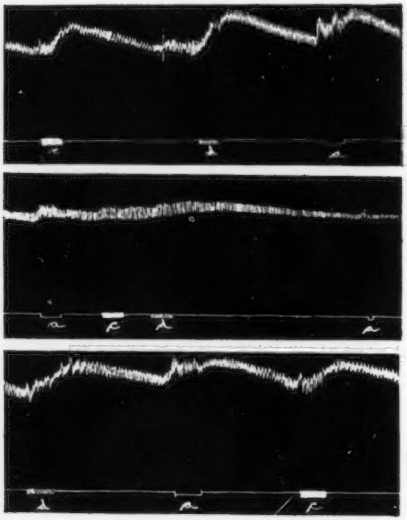


Fig. 9

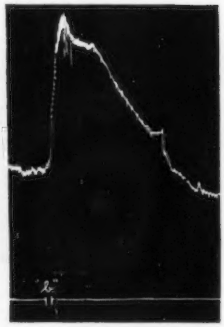


Fig. 11

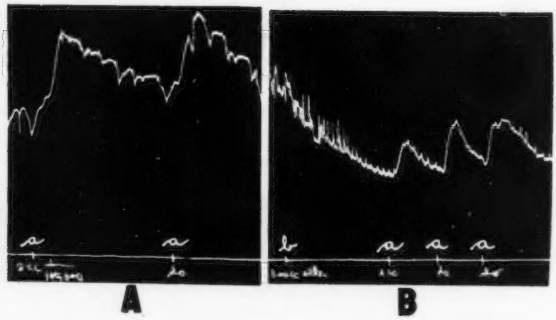


Fig. 10

Fig. 9. Blood pressure records showing the depressing effect of ether anesthesia on the pressor effect obtained by massage of various areas of the body, *c*, *d*, *a*, subcutaneously injected each with 2 cc. 1/1000 adrenalin-HCl solution (weight 5 kgm.; 6 cc. paraldehyde + $\frac{1}{6}$ gr. morphine sulphate). *A*, *c*, *d*, *a* = Massage of three areas each injected previously with 2 cc. 1/1000 adrenalin-HCl solution before the administration of ether by inhalation. *B*, *a*, *c*, *d*, *a* = Massage of the same areas as in *A* at the height of a profound inhalation ether anesthesia. *C*, *d*, *a*, *c* = Massage of the same areas following recovery from deep ether anesthesia.

Fig. 10. Blood pressure record showing a pronounced diminution in the pressor response following the intravenous injection of 2 cc. 1/100,000 adrenalin hydrochloride as a result of the intravenous injection of 1 cc. ether. (Weight of dog, 7 kgm.; no paraldehyde but $\frac{1}{4}$ gr. morphine sulphate reinforced by ether.) *A* = *a*, intra-

under this anesthetic if the dose of barbital-Na has been gauged properly. As a general rule barbitalized dogs become more profoundly anesthetized as time goes on whereas dogs under paraldehyde become lightly anesthetized in time since this anesthetic is lost to the body at each breath. Ether anesthesia as it is practiced in most laboratories will hardly do. The pressor effect once obtained in an animal under paraldehyde can be suspended during the time of profound ether inhalation anesthesia. Figure 9 illustrates the results obtained in such an experiment. On several occasions we experimented under deep morphine anesthesia using ether anesthesia solely during the preparation of the animal for the registration of the blood pressure record. Under these conditions the response on massage of an adrenalinized area was much earlier indicating that the usual anesthetic agents have a depressing influence on the myoneural junction in the arterioles.

3. *Fatigue or depression of the peripheral end organs.* The oft repeated massage of a given adrenalinized area is followed by a progressively weaker response. After ½ hour or more of rest massage of this same area again gave a marked pressor effect. We have explained this fact provisionally as an effect of fatigue of the peripheral apparatus.

What greatly surprised us, however, was the marked diminution in response of a given animal to *intravenously* injected adrenalin after intravenous injection of small amounts of ether, as shown in figure 10. A similar depression or diminution of the pressor effect seen after massage of an adrenalinized area was observed after the intravenous injection of small quantities of paraldehyde or barbital-Na.

Fate of the subcutaneously injected adrenalin. Perhaps because of the rapid deterioration of adrenalin in vitro, when exposed to atmospheric oxygen, most observers are of the opinion that adrenalin incorporated in the body is destroyed with extreme rapidity in spite of the fact that Patta had shown long ago (1901) that appreciable amounts of adrenalin could be obtained by extracting the subcutaneous tissue two hours after injecting adrenalin into it. This we can confirm. Figure 11 shows the hemodynamic effect (a rise of 150 mm. Hg) as a result of injecting a saline extract of the subcutaneous tissue 9½ hours after the subcutaneous injection of 3 cc. 1/1000 adrenalin solution following 49 pressor responses obtained from this area by massage. It would seem from this and similar experiments

venous injection of 1 cc. of ether followed by three intravenous injections of 2 cc. 1/100,000 adrenalin hydrochloride. $B = b$, intravenous injection of 1 cc. of ether followed by three intravenous injections of 2 cc. 1/100,000 adrenalin-HCl each at a, a, a.

Fig. 11. Blood pressure record showing the effect of the intravenous injection at b, of a saline solution extract of the subcutaneous tissue into which 3 cc. 1/1000 adrenalin-HCl had been injected 9½ hours previously and from which 49 pressor responses had been obtained by massage (weight of dog, 9.5 kgm.).

that in the dog a subcutaneous area injected with adrenalin becomes a veritable reservoir of available adrenalin which can be massaged into the general circulation as desired and that adrenalin so stored is not rapidly oxidized as a result of contact with the cells of the tissue which it infiltrates. As a matter of fact we have obtained pressor effects from massage 19 hours after the subcutaneous injection of 1 cc. of 1/1000 adrenalin HCl solution. Even after that period of time a saline extract of the subcutaneous tissue exerted, on intravenous injection, a marked pressor effect.

Discussion. On the basis of these data there can be no question that we were dealing, on massage of an adrenalinized area, with a pressor response due solely to epinephrin that reached the general circulation as a result of the massage and not to a vasoconstrictor reflex. Besides calling attention to the delayed response (17 seconds) following massage before the pressor effect first appeared, the abrupt rise and evanescent character of the blood pressure effect, we showed that these results could be reversed by suitable doses of ergotamine tartrate injected intravenously and, on the other hand, they may be accentuated by sufficient doses of cocaine hydrochloride. Tatum (1920) has shown in extending the work by Fröhlich and Loewi on the synergistic action between cocaine and epinephrin, using as a biological test the effect of splanchnic stimulation, that the pressor effect following stimulation of the peripheral end of the splanchnic nerve is markedly increased after the intravenous injection of 5 cc. of 0.3 per cent solution of cocaine hydrochloride.

Since the pressor effect following massage of adrenalinized hypodermic areas is usually less marked than that following intravenous epinephrin injections, we expected that cocaine sensitization would be quite manifest in our experiments. This was indeed the case in the majority of them.

We were surprised to observe that the depth and possibly the nature of the anesthetic has a marked effect on the magnitude of the subcutaneously injected epinephrin response. We could not find any data in the literature which would give an adequate explanation of this phenomenon. We may cite, however, some instances which seem to be somehwat related to it.

Collip (1921) carried out some experiments by injecting intravenously minute doses of epinephrin, which, as is well known, invariably give a depressor effect. Deep ether or $CHCl_3$ anesthesia changes these depressor epinephrin responses to pressor ones. Furthermore, he showed that the injection of 40 cc. of 20 per cent $NaCO_3$ solution also converts the depressor effects into pressor responses, whereas the pressor effects may be decreased by the injection of 10 cc. of 10 per cent solution of NaH_2PO_4.

Burget (unpublished, personal communication) conceived the idea that the magnitude of epinephrin response may be dependent upon the reaction of the blood. Over-ventilation of the lungs (alkalosis) increased the magnitude to a given dose of intravenously injected epinephrin. Under-

ventilation (acidosis) diminished the response to the same doses of epinephrin. In other words, there was a direct relation in a given animal with a given dose of epinephrin between the pH of the blood and the magnitude of the epinephrin response. We feel justified in stating on the basis of our present knowledge that the deeper the anesthetic the less pronounced the epinephrin response. It is well known that the deeper the anesthetic the greater the degree the acidosis of the blood. The variability of the epinephrin effects in relation to the nature and depth of the anesthesia might be explained on the basis of the changes in the acid-base equilibrium.

This contention may throw some light on the question of the possible state of the motor peripheral sympathetic nerve endings. Anesthetics or the reaction of the blood may influence the state of these nerve endings. In alkalosis they may be especially susceptible, in acidosis they may be depressed. The fact that the magnitude of the epinephrin response gradually diminishes after oft-repeated massage and then after ½ to 1½ hours' rest it comes back to its original level, may indicate a fatigue and a subsequent recovery of the peripheral nerve endings. The basis for such an assumption is the work by Fröhlich and Pick (1912) in which they showed that repeated injections of the quite inactive d-epinephrin diminish very markedly the magnitude of the potent l-epinephrin response. The work of Fröhlich and Pick may be explained solely on the assumption of peripheral fatigue.

Another feature of our work is the stability of the subcutaneously injected epinephrin. Most investigators assumed a very rapid destruction of epinephrin. Patta (1905) was the only one who showed that the drug is not destroyed within two hours after injection beneath the skin. In our experiments the epinephrin was not destroyed 19 hours following hypodermic inaction. This observation may be explained by the fact that in the subcutaneous areas the epinephrin has no, or very little, available oxygen; for it is protected from the atmospheric oxygen and because the oxygen consumption of the surroundings, fatty areolar tissues, is very small. The fact that we can establish an adenalin reservoir, for at least 12 hours, beneath the skin may be of some practical use.

The facts presented force us to examine the results reported years ago by Goetsch from a new angle. This investigator (1918) stated essentially that the subcutaneous injection of small quantities of adrenalin-HCl was without noticeable effect on normal human beings but led to slowing of the respiration, mild tachycardia and commonly an elevation of blood pressure of 50 mm. Hg in definitely hyperthyroid individuals together with "an exaggeration of the clinical picture of Graves' disease, especially the nervous manifestations." So striking were the results reported that the procedure was employed by a great number of clinicians to detect the presence of incipient suspected Graves' disease in patients who otherwise

failed to show the cardinal signs and symptoms of this disease. This test, now spoken of as the Goetsch test, was widely used as a diagnostic (or differential diagnostic) test. The published reports on its reliability varied. We shall not discuss them in this paper. Assuming, however, that the rate of absorption from the subcutaneous tissue is the same in man and dog, we are in a position to explain the contradictory results of such reports on the basis of the present work.

In the execution of the test no directions are given to the effect that the injected area should be massaged. From our experience in the normal dog such a massage is quite essential for obtaining the pressor effect. If the rate of absorption in man is appreciably faster than in the dog, this manœuver is not necessary but even contraindicated, for then the hemodynamic effects particularly might be so great as to be positively dangerous. There is indeed some indirect evidence to show that the absorption rates in man and dog are different; for in the dog the subcutaneous injection of 1 cc. was followed in 19 hours by a moist gangrene of the injected area. We ascribe this difference to the fact that adrenalin causes in the dog a more intense and prolonged local vasoconstriction, in fact, a vasoconstriction so prolonged that the area becomes necrotic as a result of insufficient nutrition and gaseous exchange.

If in man, where the absorption is more rapid, massage is accidentally practiced as part of the technic a more powerful positive Goetsch test might be obtained even in a normal individual than in one who has definite hyperthyroid symptoms but in whom the subcutaneous area was not massaged. The difference in the results of investigators may hinge in part upon this small variation in technic.

Closely related to this interpretation is one dealing with significance of the Goetsch test when positive (in hyperthyroid patients). We have shown that the magnitude of the response (in normal dogs) on massage and the time of its appearance depends in part on the local vasoconstrictor action of the drug. Anything which diminishes this action ($NaNO_2$ or inhalation of amyl nitrite) hastens the onset of the response and increases its magnitude. A flushed skin (vasodilatation) is not uncommon in hyperthyroid patients. Adrenalin injected subcutaneously must theoretically have less of a vasoconstrictor effect in them than in normal persons whose cutaneous vasoconstrictor nerves are even before the injection in a greater degree of tone. In the latter smaller quantities would be absorbed per unit of time than in the former. As a result the Goetsch test would naturally be negative. This explanation would imply a relationship between the amount of local vasodilatation at the time of injection rather than an increased sensitivity of the hyperthyroid patient because of the properties of the internal secretion of the thyroid itself acting on the myoneural junction of the arterioles. We are planning work in this direction on animals

as well as the effects of massage of the area on the blood pressure, heart rate and respiration in normal human individuals with and without massage.

If adrenalin remain stored in the human being as long as it apparently does in the dog so that we can speak of a veritable subcutaneous depot of it once a moderate dose is injected, it might be useful practically to employ our procedure of massage in patients in whom the symptoms of bronchial asthma recur soon after the subcutaneous injection of adrenalin given for its relief.

SUMMARY

1. Subcutaneous injection of adrenalin evokes a pressor effect in the dog provided the area is massaged. The rate of appearance, magnitude of the response and its character are described.

2. Conditions which affect the rate of appearance and its magnitude favorably and unfavorably are described and discussed. Profound anesthesia (ether, barbital-Na, and paraldehyde) militate against the occurrence of a pressor effect; whereas light paraldehyde anesthesia (or simply a profound morphine analgesia) and peripheral vasodilatation (effected by local injection of $NaNO_2$ or inhalation of amyl nitrite) lead to an early appearance of the pressor effect and a response of great magnitude.

3. Data are presented showing that the intravenous injection of small quantities of ether, paraldehyde, barbital-Na, depress the pressor effect obtained from the intravenous injection of adrenalin as well as the effect from massage of an adrenalinized area as well.

4. The fact that an increased response from massage can be obtained following a period of rest suggests that the oft-repeated massage with pressor effect leads to a depression (fatigue) of the peripheral end organ.

5. In the dog, at least, adrenalin injected subcutaneously remains there for a very long time. In spite of numerous pressor effects obtained as a result of the massage of the adrenalinized area a saline solution extract made 9½ hours following the injection of 3.0 cc. adrenalin gave, when injected intravenously, a very marked pressor effect.

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ON THE ALLEGED ANTAGONISTIC ACTION OF THE INTERNAL SECRETIONS OF THE PANCREAS AND THE THYROID

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The belief that the body's metabolism as controlled by the endocrine glands is multi-glandular has been the subject of much controversy without a great deal of proof in either direction. One of the reports concerning the alleged interdependent action was the work of Friedman and Gottesman (1) who contended that they had obtained, experimentally, an astounding diminution in the glycosuria of pancreatic diabetes in dogs the day following thryo-parathyroidectomy. They interpreted this drop in sugar excretion to the absence of the internal secretion of the thyroids. Since most of their animals, on which both operations were complete, died within a few days from parathyroid tetany, and since adequate means of control of the tetany were developed at that time by Dragstedt (2) and Luckhardt (3), it seemed advisable to repeat the work. As a thyroid deficiency is not detectable as far as the usual signs and symptoms are concerned before a week or more, the diagrammatic results of Friedman and Gottesman, purporting to show an inter-relation or antagonism of the two glands, appeared even more doubtful. It was therefore decided to repeat the work under conditions of adequate control of the tetany, on animals on a definite dietary regime, and with determinations of the blood and urinary sugars made regularly throughout the period of survival.

In reviewing the literature dealing with the relationship between the pancreas and the thyroids we were impressed with the many conflicting clinical reports of diabetes mellitus and hyperthyroidism, and the inconclusive experimental work so far done. The experimental work so far carried on lacks decisiveness because of the almost immediate onset of tetany and the subsequent death of the animal. Insufficient attention was paid the blood sugar levels, while too much emphasis was placed on the degree of glycosuria, which might easily be affected by the lowered metabolism of hypothyroidism through its renal effect, while the blood sugar might at the same time remain high.

Without going into too great detail of the clinical aspect of the question, which has been fully discussed by Allen (4) as well as Friedman and Gottes-

man, we would like to call particular attention to the detailed account of Fitz (5) in which he attributes the lowering or diminution of glycosuria to a change in the rate of metabolism and not to the fact that a portion of the gland is functionless. Wilder (6) corroborates Fitz to the effect that the evidence clinically is not indicative of a curative effect on diabetes by thyroidectomy. Many clinicians are more prone to believe that the causative agent of the glycosuria, which is not a true diabetes mellitus, is an increased body metabolism resulting from a hyperfunctioning thyroid.

EXPERIMENTAL PROCEDURE. Only dogs were used in this work. The experimental procedure was to place the animals on a routine diet of 150 grams of beef hearts and 225 cc. of milk, as a preliminary control period. Normal blood and urinary sugar determinations by the method of Folin-Wu were made for three days preceding the pancreatectomy. The animals were then totally depancreatized in order to avoid all possibility of pancreas regeneration as may occur in partial removal of the pancreas. The blood and, when possible, urinary sugars were determined for twenty-four-hour periods, the samples being taken just before feeding. This routine was followed for a period sufficient to determine the condition of diabetes, and was followed by the removal of the thyroids. Whenever the parathyroids were not in the substance of the thyroid they were left behind. The day following the second operation the dogs were placed on the routine diet plus 15 to 20 grams of calcium carbonate and calcium lactate daily, in order to remove the tetany factor in which we were not particularly interested. In this manner we were able to keep the animals alive for some time following the second operation.

RESULTS. Total pancreatectomy was followed at a later period by thyroidectomy. Eight dogs were used. One of these died during the thyroid removal; the other died eight hours following the removal of the thyroids. A complete protocol of one of the dogs (table 1) and graphs of two of the animals (figs. 1 and 2) are representative of the results. A summary of the protocols of the six animals follows:

Experiment 4 (table 1). Female, weight 13.8 kilos. Blood sugar values of 80.6 to 85.8; 100.4 mgm. of sugar per 100 cc. of blood with no sugar in the urine for three days preceding the operation. 11–6–25, total pancreatectomy was performed. For ten days following the operation the blood sugar varied from 280 to 350 mgm. per 100 cc. and there was considerable sugar in the urine. 11–17–25, complete thyroidectomy was performed. For the following seven days the blood sugar varied from 390 to 480 mgm. per 100 cc. and there was still sugar in the urine. Animal died November 24, weighing only 8.3 kilos at the time of death.

Experiment 5. Female, weight 16.8 kilos. Normal blood sugars of 80 and 83.2 mgm. per 100 cc. 12–3–25, total pancreatectomy was performed. The day following the blood sugar was 220 mgm. and rose steadily to 333 mgm. December 9, the thyroids were removed. The blood sugar was 493 mgm. the following day and the animal died on the second day after operation. No urine was collected from this animal, so quantitative determinations were not made.

Experiment 6. Female, weight 17.1 kilos. Normal blood sugars 83.3 to 79.0 mgm. per 100 cc. December 16, total pancreatectomy was performed. From December 16 to 28 the blood sugar rose to 227 and 292. On the 28th the thyroids were removed. For three days following the blood sugar levels were 417, 435, 465 respectively and the animal died on the fourth day.

TABLE 1

Complete protocol for experiment 4

DATE	WEIGHT	BLOOD SUGAR PER 100 CC.	VOLUME URINE	SUGAR IN URINE	CONDITION
	kgm.	*mgm.*		*grams*	
11–4–25	13.8	80.6	–	–	Very good—young animal—placed on diet of 250 grams beef heart—225 cc. milk
11–5		85.8		–	Starved
11–6		100.4			Totally depancreatized
11–7		none taken		+ with 3 drops	Good—given little water
11–8		327.0		+	Drank and retained 100 cc. milk
11–9		305.4		+	Restored to normal diet
11–10		305.3		+	Animal in good condition but drowsy
11–11		290.0	1250	27.5	O. K.
11–12	11.4	323.0	1130	28.3	O. K.
11–13		296.3	1320	31.4	O. K.
11–14		283.7	710	18.3	O. K. but becoming very thin
11–15		294.8	1470	35.4	O. K.
11–16					O. K.
11–17	9.8	367.0			Removed both lobes of the thyroid
11–18		430.1			Fine recovery—15 grams Ca lactate added to food
11–19		476.2	1150	31.0	O. K.
11–20	9.0	392.2	624	16.9	O. K. but so weak and emaciated is unable to stand
11–21		408.1	850	20.4	O. K. but must be fed due to extreme weakness
11–22		444.0	800	19.2	
11–23	8.3	400.2			Found blood in cage—secondary hemorrage in neck—stopped
11–24					Animal found dead from another hemorrage—same place

Experiment 9 (fig. 1). Male, weight 13.6 kilos. Normal blood sugar 79.4 to 88.6 mgm. per 100 cc. Pancreas removed April 19, 1926, and the blood sugar rose from 216 mgm. the day following the operation to 291 mgm. on the 29th. There was a great deal of sugar in the urine throughout this period. On the 29th the thyroids were removed. For five days following the sugar varied from 275 to 290 mgm. On May 4 forty units of insulin was given the animal and on the 5th the sugar was at 70 mgm. and forty more units were given with a meal rich in carbohydrates. Weight of the animal at this time was 8.65 kilos. The following morning the animal was found dead.

Experiment 10 (fig. 2). Female, weight 13.4 kilos. Normal blood sugar was 85, 104, 88 mgm. May 1, 1926, the pancreas was removed. From the 2nd to the 8th the sugar rose daily from 184 to 287 mgm. per 100 cc. and there was a large amount of sugar in the urine. Weight at this time was 11.1 kilos. On the 8th the thyroids were removed, following which the blood sugar remained at about 275 mgm., and there was considerable dextrose in the urine. The animal died on the 13th. Weight just prior to death was 10.1 kilos.

Discussion. Unlike the results of Friedman and Gottesman, we obtained no disappearance of the signs and symptoms of diabetes following thyro-parathyroidectomy. The blood sugar levels of our animals remained either at the same diabetic level (figs. 1 and 2) or showed a slight increase (table 1), but in no case did we find that there was a tendency for the hyperglycemia to diminish. In the work of Lorand (7) and MacCallum (8) no blood sugar determinations were made; and in that of Friedman and Gottesman only one blood sugar determination was made during the period of survival. In some cases they report a return to normal while in other cases they also find that there was no drop in sugar content. However, the inaccuracy of their reports as to the extent of their pancreatectomies and the fact that they do state that some of their animals were only partially depancreatized leads us to believe that in those cases where the sugar level returned to normal there may have been only a transient diabetes. That this may occur was long ago shown by Von Mering and Minkowski, who by removing all but a small portion of the gland obtained recovery and normal conditions in a short time, due mainly to a regeneration of pancreatic tissue.

Unlike the blood sugar, the daily excretion of sugar in the urine of our animals showed a slight change. During the period preceding the thyroidectomy the urinary sugar showed some fluctuations (figs. 1 and 2), but after thyroidectomy there was a slight drop which continued until death though we failed to obtain a condition of aglycosuria at any time. MacCallum obtained only a diminution in the glycosuria of his totally depancreatized animals and a total disappearance of sugar in his partial pancreatectomy. Likewise Friedman and Gottesman have obtained results of both characters. On the basis of our work and the high sugar levels we do not feel that the diminution in the sugar secretion is due to an antagonism of the two glands. We believe that the lowered sugar output is a result of the lowered rate of body metabolism rather than a curative effect. The "slowing of metabolism" due to thyroid deficiency was proven early by Marinesco and Parhon (9). As Allen pointed out, "glycosuria is not a measure of diabetes" and there is no evidence that the animals in these experiments were any better able to "utilize" sugar. In fact the animals of Lorand, MacCallum, and some of those of Friedman and Gottesman died very shortly after the second operation. They were

commonly in such a condition of cachexia, weakness and shock, that their moribund condition itself might account for the disappearance of sugar in the urine. When our animals became moribund we also noted a diminution in the glycosuria.

Clinically, the coincidence of diabetes and hyperthyroidism has been occasionally reported, but the preponderance of evidence seems in favor of the rarity of this combination (Wilder, 1.1 per cent). Probably no other reports show as complete an analysis of this condition as those of Fitz and Wilder. Fitz reports a series of thirteen cases of diabetes; four with non-toxic goiter and nine with toxic goiter. In the first group partial

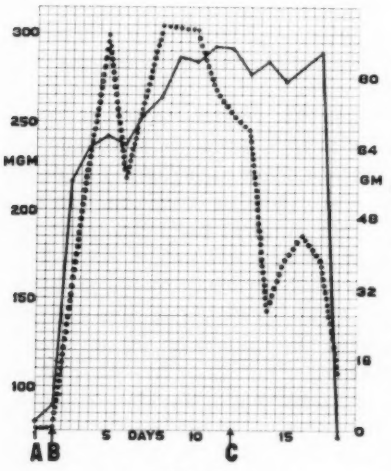

Fig. 1

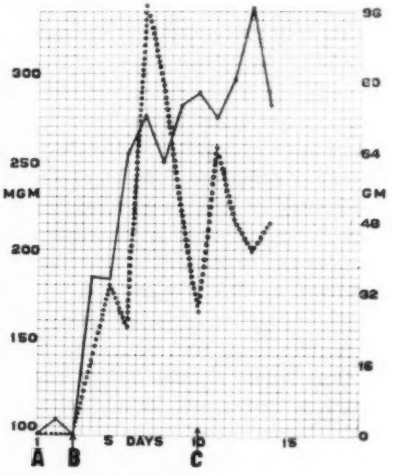

Fig. 2

Fig. 1. Showing in solid line (▬) the blood sugar level in milligrams per 100 cc. in experiment 9, as well as the glucose content of the urine (●●●) in grams per day. *A* = before pancreatectomy; *B* = pancreatectomy; *C* = thyroidectomy.

Fig. 2. Same as figure 1 for experiment 10.

thyroidectomy was performed; three cases showed no improvement of the diabetic conditions, while one case returned to normal and remained sugar free if strict dieting was maintained. In the second group the thyroids were completely removed; six cases were unimproved as far as the diabetes was concerned while three of this group showed a condition of aglycosuria. However, as was previously pointed out, he attributed this rather to a change in the rate of metabolism than to any specific interrelationship of the two glands. Wilder, in a recently published report (6), shows that, since the discovery by Banting and Macleod of insulin, patients of this type may be much more satisfactorily operated upon and with a

minimum of post-operative deaths if they are given the proper pre-operative treatment to have them sugar free at the time of operation. He attributes some of the negative results of Fitz and others to this factor and himself cites cases of extraordinary beneficial results.

If an antagonism of the two glands actually existed we might expect a greater coincidence of diabetes and hyperthyroidism clinically. Furthermore, Garrod (10), Lepine (11), Magnus (12) and Opie (13) have all reported cases of the simultaneous existence of diabetes and thyroid deficiency, which on this hypothesis would be unexplainable. The probable explanation, however, of the coincidence of hyperthyroidism and diabetes is that due to the nature of the disturbance when either of the two glands is involved the individual is more susceptible to involvemnt of the other gland than is a normal case. However, the recent work of Wilder leaves us in doubt as to the efficiency of our own experimental attempts as well as those of the other investigators. We find that we are somewhat in the position of Fitz prior to the discovery of insulin, since we, as well as the other investigators, failed to take cognizance of preparing our animals by pre-operative treatment when they were in a diabetic condition. In this way our percentage of fatalities and poor surgical risks ran high, and some of the experiments were therefore of questionable value. This does, however, seem to indicate a new method of approach to the problem. Furthermore, we are not sure of the exact condition of our animals after thyroidectomy, as it has never been determined as to the length of time which elapses before any of the other effects of hypothyroidism manifest themselves in the dog. On the other hand, our data collected under better experimental conditions than those of Friedman and Gottesman and on the same animal (dog) failed to support the immediate and marked beneficial results of thyroidectomy on the course of pancreatic diabetes reported by them.

The results of the experimental studies so far carried out are very inconclusive and poorly controlled. The clinical evidence, particularly that of Wilder, shows some favorable effects following thyroidectomy in patients suffering from hyperthyroidism and diabetes. Whether or not a subtotal thyroidectomy in an individual having diabetes and normal thyroids would be beneficial is a matter of conjecture. Nevertheless, the question is still an open one offering several lines for further experimental work which might be of clinical significance.

SUMMARY

1. Thyroidectomy on diabetic animals without insulin treatment is ineffective in lowering blood sugar levels during the recorded period of survival of the animals.

2. Urinary sugar excretion may be somewhat diminished, but the animal is never aglycosuric.

3. The experimental procedure employed, while an improvement over the former methods, is still lacking in complete control.

4. A state of hypothyroidism in the dog has never been completely established.

5. Further work should be done under methods of even better control.

I wish to express my deep appreciation to Doctor Luckhardt for his many valuable suggestions and aid in preparing the manuscript for publication. I also wish to thank Miss Dorothy Koch for the able surgical assistance she gave in this work.

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THIRTY-NINTH ANNUAL MEETING

Rochester, N. Y., April 14, 15, 16, 1927

Automatic contractions of isolated strips of mammalian heart muscle. C. W. GREENE and R. W. SIDDLE.

We have used hearts of young dogs and cats to make cardiac strips of the Gaskell type. Similar experiments were done by Erlanger, by Morehouse, by Taussig and Meserve. The latter especially have cut strips from a wide variety of regions of the heart. We have made strips from the auricle, cutting in such a way as to include portions near the sinus node, or of the sulcus,—from the atrium, from the intra-auricular septum and from the left auricle; also from the outer wall of the right ventricle, the intraventicular septum, from the left wall both outer and inner surfaces, and of papillary muscles. The ventricular strips were longitudinal and many contained Perkinje tissue. The atrium of course does not contain this tissue. We suspended these strips as quickly as possible in dextrose Ringer's solution, pH 7.2 and aerated with atmospheric oxygen.

Automatic contractions occurred in a fair percentage of the preparations, but usually slow irregular rate and of varying amplitude. These contractions never endured for long.

When a small quantity of adrenalin, as used by Taussig and Meserve, was added, after a longer or shorter latent period, a rhythm was established of wonderful regularity and equivalent to normal rate amplitude. This rhythm continued often for five or six hours. The rate and amplitude were highest, immediately after the rhythm was established by adrenalin stimulation, but settled back somewhat to a very constant rate and amplitude. In the auricle this rate was from 90 to 130 per minute. In some cases remained without change for even as much as two hours. The rate was comparatively lower in the ventricle and did not last so long. Both auricular and ventricular strips were restimulated to further amplitude and increased rate on successive additions of adrenalin.

We have used other drugs but we mention only calcium chloride which when doubled in quantity stimulates a similar rhythm in quiescent strips.

We lean more and more to the view, as the experiments have accumulated, that adrenalin stimulates either through the accelerator nerve endings, or by means of the Perkinje tissue. This view is based in part on the observed greater rhythm in portions which contain endothelial hence Perkinje tissue. In fact, there is an obvious gradient in strips from the same heart. Auricular tissue containing sinus or sulcus tissue from the right ventricular wall, and the capillary muscles are the easiest to stimulate, whereas it is difficult to stimulate atrium to a regular rhythm.

Further comparative work is in progress.

The contraction type in the isolated uterine horns of the rat. C. W. GREENE, T. W. EDMONDS, O. W. CRAIG and HAROLD H. GREENE.

The uterine horns of the rat are very favorable in size and development

for isolation studies. The vascularity and size, and apparently the irritability, of this tissue varies with the estrous cycle. The isolated uterine horn, in glucose Ringer's solution at 34 to 36°C. and fully aerated, contracts with a slow, regular and dependable rhythm. The contraction wave begins at the ovarian end and travels over the tube in a simple peristalsis. Each contraction wave does not always cover the whole length of the tissue which produces some degree of irregularity.

The normal contractions will continue by the hour in this isolated tube. The preparation is very sensitive to a great variety of drugs. Of these, we mention here only two. Adrenalin produces a quick and pronounced inhibition in dilutions of 1 in 10 million. Inhibition is produced in the uterus when in all stages of its development. In the gravid uterus in which the embryos are approaching full term prompt inhibition of the muscular wall occurs as definitely as in any non-gravid preparation.

Solutions of cocaine, novocaine, tutocaine, all as chlorides, produce reactions in solutions diluted to 0.002 per cent. In this dilution the uterus is stimulated to strong tonic contraction with prolongation of the contraction phase. If the drug is increased in concentration to 0.1 or more per cent, then pronounced relaxation and inactivity result. We have not looked on this as an inhibition but as a narcosis. Recovery is slow but complete. In the after-period of cocaine poisoning, the tissue is thrown into pronounced tonic contraction with persistent rhythm, usually at a higher rate.

Crystalline insulin. JOHN J. ABEL.

The methods employed in crystallizing insulin with the recent improvement will be described. The question as to whether the crystals prepared hold attached to them by adsorption a more powerful substance will be discussed. The chemical reactions of the crystals and their physical properties, such as their optical activity in various media; the molecular formula of the compound, the amount of labile sulphur present and other points, will be considered. Questions pertaining to animal tests with the product will also be taken up.

A quantitative description of the adaptation of frogs to the concentration of their medium. EDWARD F. ADOLPH.

Frogs of various weights were transferred to solutions of sodium chloride of diverse concentrations. At known times the weights of the body, amounts of urine formed, freezing points of muscles, chloride contents of the body, and chloride contents of the urine were measured. From these data, which are based on average values for many individuals, the following ten quantities may be compared with each other: body size, time, concentration of medium, concentration of tissues, water content of body, rate of water excretion in urine, rate of passage of water through skin, rate of passage of chloride through skin, rate of chloride excretion in urine, and osmotic pressure difference between tissues and medium. For frogs of a given size, only two of these quantities (time and concentration of medium) are independent variables, and the other seven quantities are dependent variables. Taking the independent variables as coördinates, each of the other seven quantities may be represented by contours. These contour charts (nomograms) have been constructed. From them can be read off the particular quantities which represent the average amounts of

modification undergone at any moment by frogs in response to the medium. For certain cases other pairs of variables have been used as coördinates in further charts.

If the assumption be made that the difference of osmotic pressure between tissues and medium is proportional to the rate at which osmotic force alone would move water through the skin of the frogs, then the difference between the rate at which the water is actually passing through the skin and the rate due to this one force represents a measure of other force or forces acting at the skin. The relative magnitudes of such force or forces at various times have also been graphed. In no case do the latter magnitudes continue to be zero.

Myrtillin. FREDERICK M. ALLEN.

Myrtillin is an extract of green leaves, prepared according to a method discovered and described by Dr. R. I. Wagner. Teas or powders of leaves of the myrtle family of plants have long been used for treating diabetes among the peasants in certain regions, and for technical reasons these plants are actually the best source of the material in quantity. It is called myrtillin for this reason. The chemical nature or composition is unknown; it may possibly be classifiable among the vitamins. It is non-toxic. It is entirely different from insulin and all insulin-like substances obtained heretofore from animal or vegetable sources. It is effective when given by mouth. It has no important influence upon the normal fasting blood sugar, and never causes hypoglycemia, tending rather to prevent hypoglycemia due to insulin. Its prominent physiological properties, according to experiments performed in collaboration with Doctor Wagner, with doses of 0.3 to 2.0 grams per day, are as follows:

It reduces the glycosuria and hyperglycemia following glucose administration (orally or intravenously) in normal animals or persons.

It reduces adrenalin hyperglycemia.

It accelerates oxygen consumption after carbohydrate ingestion in normal and diabetic persons, indicating a probable stimulation of the combustion.

It enables totally depancreatized dogs to heal their wounds and live 4 to 6 weeks in good condition, fatal cachexia then ensuing. These dogs have marked hyperglycemia and moderate glycosuria, but on proper diet the carbohydrate balance is positive. Glycogen values may be 3 or 4 per cent in the liver, and are within normal limits in the heart.

Dogs not quite totally depancreatized, retaining one-twentieth or less of the pancreas, are enabled by myrtillin to be free from glycosuria or hyperglycemia on diets containing liberal carbohydrate, and they apparently thrive indefinitely.

The results in diabetic patients are more gradual and less uniform, and are to be described in later communications.

Myrtillin is not a substitute for insulin, but is probably to be regarded as an accessory substance which is widely distributed in nature and which plays a part in the carbohydrate metabolism of plants and animals. It thus has a distinct therapeutic usefulness when properly administered to selected diabetic patients.

The relative duration of contraction in flexors and in extensors. P. C. BAIRD, JR. and J. F. FULTON.

Accurate optical myographic registration of the motor nerve twitch of

flexors (*e.g.*, semitendinosus) and extensors (*e.g.*, vastocrureus) of the knee- and ankle-joints of decerebrate cats has shown that the duration of contraction is consistently less in the flexors than in extensors. For example, the duration of twitch of semitendinosus, a knee flexor, lies in various preparations between 25 and 32σ, while that of the vastocrureus ranges from 35 to 43σ. This is regarded as significant since the chronaxie of the nerves (motor points) to these muscles (as determined by Buchanan and Garven, Journ. Physiol., 1926, lxii, 120) vary similarly, that of semitendinosus being 0.11 to 0.16σ and that of the knee extensor $\pm$0.33σ. Similar observations upon the duration of contraction of flexors and extensors have been made upon the muscles of the tortoise by Bremer and Cambier (*C. R. Soc. Biol.*, Paris, 1925, xciii, 61).

Metabolic exchange in certain snakes at 20°C., with notes on size and seasonal differences: (Pituophis sayi or Bull-snake) and (Lampropeltis getulus holbrooki or King-snake). F. M. Baldwin.

Using the method of indirect calorimetry under temperature control, several series of observations were obtained on the oxygen consumption in two of the common snakes of Iowa. In the first series on the bull-snakes, extending over the period from February to May (1926) the smallest snake weighing 422 grams (average) consumed oxygen at an average rate of 0.0740 cc. gram hour, while the largest weighing 822 grams (average) consumed 0.0425 cc. gram hour. The average consumption for all specimens in this series was 0.0583 cc. gram hour. Although at first sight these figures would seem to indicate that the *law of surface area* might apply, individual differences were so varied that no causal relation between surface and energy exchange can be drawn. In another series involving the king-snake, over the same time interval, similar results were obtained. The highest average rate of oxygen consumption, 0.0618 cc. gram hour, was from a specimen weighing 221 grams and the lowest rate of 0.0291 cc. gram hour, was from a specimen weighing 319 grams. Data on six bull-snakes beginning in October and extending through December (1926), furnish the basis for comparison with the figures cited for the early spring months. Figured on the same basis the highest consumption amounted to 0.0829 cc. gram hour, and the lowest in the series, 0.0301 cc. gram hour. The average for all during this interval was 0.0580 cc. gram hour, which was approximately the same as shown in the first series. Seasonal differences in consumption are apparently of no consequence when temperature is controlled. Work involving the hibernation period as well as sex differences in these forms is being carried forward.

Fasting anhydremia and phlorhizin hydremia: two conditions associated with retention of injected fluid by the blood. H. G. Barbour and R. W. Frankmann.[1]

The blood of dogs fasted five days or longer, exhibits a 5.5 to 11 per cent loss of fluid as judged by density determinations with the falling drop method. But hydremia (2.4 to 9.5 per cent) is seen instead of anhydremia, if the fasting be accompanied by a course of phlorhizin injections adequate to produce glycosuria and fatty liver. If the phlorhizin injection be

[1] This investigation has been assisted by a grant from the Ella Sachs Plotz Foundation.

delayed until after the development of anhydremia, the shift to the hydremic side of normal is striking.

Studies of the effects of intravenous injections of fluid upon the blood concentration of dogs have hitherto been held impracticable without anesthesia. We have had satisfactory results in trained dogs after an introductory rest of one hour. The standard fluid injection consisted in 25 cc. per kilo of dextrose-free Locke solution. After an average maximum dilution of about 15 per cent, the effect was reduced to 6 per cent in 15 minutes and 2.3 per cent in 60 minutes, in normal dogs.

Fasting dogs exhibited such a delay in recovery that a 9.8 per cent dilution persists after 15 minutes, and 6 per cent after 60 minutes. Almost identical figures are obtained in phlorhizinized animals. Hypoglycemia if present does not alter this result.

The above findings are based on fourteen experiments in five dogs.

Since the result of the injection of fluid is the same whether the blood is originally dilute or concentrated, the phenomenon must be regarded as refusal of storage by the tissues. Fasting acidosis may be regarded as a significant factor as well as possible changes in the fat metabolism.

Simplification of the Van Slyke-Neill manometric blood gas apparatus. GEORGE H. BISHOP and NARCISO CORDERO.

The present model is an attempt to simplify the Van Slyke-Neill[1] manometric blood gas apparatus without detracting from its accuracy. It has been used by us and by White[2] with gratifying success. The new features are believed to offer the following advantages:

1. Absence of any rubber connection within the vacuum system. The manometer and extraction chamber, as separate units, are joined to a basal unit, which puts the two into communication with each other. The junctions between the manometer and extraction chamber on the one hand, and the base on the other, are ground glass mercury-sealed joints. The evacuation preliminary to introducing the materials for analysis takes much less time than in the original model. The separation of the vacuum system into three readily detachable units makes replacement of broken parts easy and minimizes repair troubles and costs.

2. Elimination of the levelling bulb, which is substituted by a stationary reservoir, from which mercury is led to the vacuum system by suction from an ordinary water pump.

3. Elimination of the lower arms that reach almost to the floor. The whole compact outfit is about 33 inches high and can be set on any table or carried without difficulty from place to place.

In our modification it is necessary to shake the whole outfit, which, because of its compactness and small size, can be easily swayed back and forth in upright position. We realize that the shaking here is not as convenient as in the original model. However, in its present form, we feel that its advantages over the original more than outweigh this shortcoming. The details of construction and additional improvements will be described later.

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[2] White et al., This Journal, 1926, lxxviii, 185.

The potential wave accompanying contraction in skeletal muscle. G. H. BISHOP.

The action potential of skeletal muscle, like that of heart muscle, ends as late as the end of the phase of contraction; for frog sartorius removed from the body, lasting at 20°C. about 40 sigma. The action potential as usually recorded represents only the first portion of this. On long, parallel-fibered muscles where diphasic leads can be sufficiently far apart to separate the two phases, a negative T wave appears as in the slower heart muscle strip. The R and T waves of skeletal muscle are then the resultant or algebraic sum of two prolonged potentials at the two leads, whose effects balance to a plateau between these waves. This plateau may approach the base line, or may lie above or below it, as may the T wave, depending upon the activity under the two leads, and upon the time intervals between the records of the two phases.

It is impossible to obtain a strictly monophasic lead for muscle, for reasons discussed in a previous paper on nerve (Bishop, Erlanger and Gasser, 1926), but it may be approximated in several ways to be described in detail later.

Under such conditions, the nearly monophasic wave appears as an initial rise lasting in frog sartorius from 1.5 to 3 sigma, followed by a similar fall part way to the base line, this in turn followed by a further fall having a much flatter slope and lasting 20 to 40 sigma. Theoretical diphasic curves constructed from the monophasic curves recorded by means of the cathode ray oscillograph agree in detail with the diphasic curves as recorded from the usual leads, in which the late portions of the waves nearly balance out. T waves in skeletal muscle reported in the literature may be similarly interpreted.

The influence of sodium bicarbonate on the gastric response to histamine. T. E. BOYD.

In previously reported experiments a standard meal was used by the author to measure the effect of alkalies on gastric secretion. Histamine in fixed dosage has an effect less variable than that of food, and has been substituted as a stimulus to secretion in the present study.

The response of the Pavlov pouch to histamine is not affected appreciably by the previous administration of 200 cc. of 2.5 per cent $NaHCO_3$ solution, by mouth, to a 10 kilo dog. The same volume of a 5 per cent solution depresses secretion, provided water is withheld. When water is given ad libitum the secretion is normal in amount and in acid content. It seems, then, that a diminution in available water accounts for the depressing effect on secretion of concentrated $NaHCO_3$ solutions. A similar reduction in the response to histamine occurs after giving concentrated solutions of $MgSO_4$.

A possible direct effect of $NaHCO_3$ by contact with the gastric mucosa was investigated. In animals trained to lie quietly, the Pavlov pouch was irrigated by a slow stream of $NaHCO_3$ solution siphoned from a container kept at 38°C. At the end of one hour the alkali was washed out with water and the pouch allowed to drain for ten minutes. The standard dose of histamine (1 mgm. of the dihydrochloride) was then administered. The average response was practically the same as in the control experiments.

It is concluded that $NaHCO_3$, in doses up to 1 gram per kilo body weight, has no local or systemic action that affects the response of the gastric glands to histamine, except as the water supply may be modified.

The normal pulmonary circulation time in man. HERRMANN BLUMGART and SOMA WEISS.

Observations on the normal pulmonary circulation of man are presented for the first time. Sixty measurements of the pulmonary circulation time together with determinations of the vital capacity, venous and arterial pressure and pulse rate have been made. An active deposit of radium is injected into the cubital vein of one arm. When the active deposit reaches the right chambers of the heart the gamma rays produce an ionization current which, when amplified, is automatically registered by an appropriate detecting device. Similarly, the time of arrival of the active deposit in the arterial vessels about the elbow is automatically recorded. The time that elapses between the instant of injection and the time of arrival of the active deposit in the right chambers of the heart measures the velocity of venous blood from the arm to the heart. The time that elapses between the arrival of the active deposit of radium in the right heart chambers and the arrival in the arteries about the elbow of the arm measures the pulmonary circulation time plus the time lost in the heart and the time of transit of the active deposit from the left chambers of the heart to the antecubital arteries. Since the arterial velocity is, in general, twice that of the venous velocity, the measure of the venous velocity of blood to the heart offers an index of the time of transit of the radium active deposit from the heart to the antecubital arterial vessels.

The average actual pulmonary circulation time in normal resting male individuals is 6.5 seconds with a range of from four to eleven seconds. The average velocity of the venous blood to the right heart is 5.7 seconds.

Comparative measurements of the circulation in man with carbon dioxide and with ethyl iodide. R. J. BROCKLEHURST, H. W. HAGGARD and YANDELL HENDERSON.

Current textbooks still assert that the blood circulates so slowly that the arterio-venous oxygen difference is 7 or 8 volumes per cent. On the contrary, recent evidence indicates that the circulation is twice so rapid and that in a healthy man at rest the arterio-venous oxygen difference is therefore only 4 volumes per cent or less. As the relation of the circulation to the oxygen consumption of the body determines the pressure of oxygen in the tissues, every line of evidence bearing on the matter is important. The evidence presented here shows that results from the ethyl iodide method agree satisfactorily with those from the CO_2 method. The latter method was used in its simplest form: Assuming (as all available data indicate) that a, the CO_2 dissociation curve of the blood has a slope of 3.42 volumes per cent for a rise of pressure of one per cent of an atmosphere, we determined b, the arterial CO_2 pressure by the procedure of Haldane and Priestley, c, the venous CO_2 pressure of fully oxygenated blood by means of a Plesch bag containing oxygen and CO_2 following the procedure of Henderson and Prince, and d, the CO_2 production of the body. The figures from b and c are applied to a and the arterio-venous CO_2 difference thus obtained is then divided into d to calculate the circulation.

Medulliadrenal secretion and carbohydrate metabolism. S. W. Britton and E. M. K. Geiling.

Since the introduction of insulin much discussion has arisen regarding the function of the adrenal medulla in its possible relationship to carbohydrate metabolism. Inquiry was made into this problem in the present series of experiments. After having carried out control observations on the response of normal dogs and cats to insulin, medulliadrenal inactivation was performed by the operation of *a*, medullectomy and also by that of *b*, removal of the right adrenal gland and denervation of the left. Both types of operated animals, maintained in vigorous health, exhibited striking changes in susceptibility to insulin. Whereas the hypoglycemic reactions were commonly slight or absent on administering two or (sometimes) four units of insulin per kilo weight before operation, lower blood sugar values and convulsive seizures were often observed following the injection of 0.5 or (sometimes) 0.2 unit per kilo weight into the same animals after the adrenal medulla had been rendered nonfunctional. The suppression of adrenin secretion in animals therefore resulted in a four to ten-fold increase in sensitivity to insulin. The depression of the sympathetic nervous system by the administration of ergotamine further, but only slightly, accentuated this hypersensitivity. During hypoglycemic prostration in these cases there occurred convulsive fits, interposed by general tonico-clonic contractures, and manifesting a tendency to become confluent in character. The adrenal inactivated animals showed no increase in blood sugar immediately following convulsions. That the glycogen reserves were adequate could be demonstrated, however, by the fact that small amounts of epinephrin rapidly elevated the blood sugar and promoted recovery. Corresponding increments in the glycemia did not occur on giving adrenalin to convulsant animals with adrenals intact. The increased sensitivity to insulin of medulliadrenal inactive animals has persisted for over three months. Complete protection against the lowering of the blood sugar percentage and insulin shock was nevertheless readily afforded by the administration at suitable intervals, commencing shortly after the administration of insulin, of small doses of adrenalin. Derived from this evidence are the conclusions that a highly potent secretion from the adrenal medulla, tending towards depletion of the glycogen stores in the body while supplying sugar to the blood stream for use by the important (e.g., nervous and muscular) tissues, takes place during insulin hypoglycemia, and that in the absence of this secretion the glycogen depots although well furnished have a much restricted availability (and that through the medium of the sympathetic nervous system) to the interdependent parts of the organism.

The tolerance of excised muscle toward variations in the calcium content of the fluid medium and preliminary findings on the effect of parathyroid extract. E. H. Brunquist.

The work is presented as of interest in leading up to the general question of the extent to which excised muscle can retain its contractility for 12 hours or more under various adverse conditions, and of the relation of the calcium content of the medium to such tolerance.

The frog's sartorius was used, and data are presented showing the very considerable tolerance of the tissue toward changes in the proportion of

calcium in Ringer's solution. Preliminary findings concerning a possible effect of parathyroid extract (Collip; Lilly preparation) on this tolerance are presented.

Calcium deficiency and bone changes in experimental obstructive jaundice. WM. C. BUCHBINDER and RUTH KERN.

Further observations of the calcium content of the blood serum of puppies with experimentally induced jaundice reveal a fairly progressive decrease which substantiates the findings previously recorded. Although the blood serum calcium was reduced to the tetany level, no sign of increased irritability of the neuromuscular apparatus was noted. A marked osteoporotic condition with marked thinning of the cortical portions of the bone and increased fragility was a constant finding. The further striking observations were that the survival period after the induction of jaundice was much longer than that hitherto recorded for the adult animal. Growth of skeletal structures with a considerable gain in body weight was noted at the time of death.

The factors responsible for the bone lysis may be the rapid growth and failure of calcium absorption in the presence of some calcium precipitating mechanism by certain of the biliary constituents. A parathyroid element is suspected of playing some rôle and the evaluation of this factor may be made possible by noting the effects of parathyroidectomy in the recently and chronically jaundiced animals, both young and adult, experiments which are now being carried out in this laboratory.

The effect of the internal secretions and temperature on the metabolism of amino acids and simple sugars by animal cells. W. E. BURGE, A. M. ESTES, G. C. WICKWIRE and MAUDE WILLIAMS.

The internal secretions used in this investigation were insulin, thyroxin, adrenalin and pituitrin and the sugars dextrose, levulose and galactose. The amino acids used were glycocoll, tyrosin, nor-leucin, iso-leucin, cystin and a mixture of the naturally occurring amino acids, aminoids. Benedict's method was used for making the sugar determinations and Van Slyke's and Sörensen's for the amino acid determinations.

Large quantities of paramecia were collected, washed free of debris by centrifugalization and measured in centrifugalizing tubes which were graduated in cubic centimeters. Paramecia sugar preparations were made with 5 cc. of paramecia in 100 cc. of 0.1 per cent sugar solutions. Amino acid and aminoid paramecia preparations were made with 5 cc. of paramecia in 0.3 per cent solutions of these materials. Various amounts of insulin, thyroxin, adrenalin and pituitrin were added to these preparations and sugar determinations were made immediately and subsequently at certain intervals.

Paramecia use the three simple sugars dextrose, levulose and galactose. They use dextrose and levulose more rapidly than the galactose. Insulin increases the utilization of all three of these sugars just as it does in the higher animals. Pituitrin also increases the utilization of these sugars while thyroxin decreases it. Small amounts of adrenalin increase and large amounts decrease the sugar metabolism of these organisms.

Paramecia use the amino acids and the aminoids, but not nearly so rapidly as they do the sugars. The addition of insulin and pituitrin have

no effect on the metabolism of the amino acids while adrenalin and thyroxin increase it slightly.

Paramecia metabolize practically no sugar at 0°C. and the metabolism is increased with a rise in temperature.

Further observations on the depressor substance in liver extract. THEO. C. BURNETT.

Both dried and fresh liver have been extracted with absolute alcohol for twelve hours in a Soxhlet's apparatus, after the method described by Sharpey-Schafer and MacDonald for the posterior pituitary. The extract contains the depressor principle, while an extract of the liver residue is inert. As histamine is said to be removed by the absolute alcohol and as extracts of the residue are inert it would seem that the depressor substance is histamine, although it is admitted that a specific secretion might be soluble in absolute alcohol. Nevertheless, clinical reports seem to show that liver extract is more satisfactory in treating hypertension than histamine or choline or a combination of the two.

The influence of nicotine and caffeine on the growth of chickens. CHARLES E. CHASE.

Large doses of nicotine were introduced daily into the crops of growing chicks by means of a pipette and records of their weights taken to determine whether the growth curve was affected in comparison to that of controls to which an equal amount of water was administered in the same way. A second line was given caffeine (alkaloid) in this manner.

Our results indicate that nicotine in large doses first stimulated growth and later retarded it.

Caffeine retarded growth throughout the course of the experiments.

Protein metabolism during muscular exercise. WILLIAM H. CHAMBERS and ADOLPH T. MILHORAT.

Dogs have been exercised daily for three consecutive half-hour periods by running on a motor-driven treadmill at a constant rate of four miles per hour. The total nitrogen excreted in the urine was determined for several periods before, during and after the work.

The nitrogen metabolism depended on the state of nutrition of the animal. In fasting animals which had previously been well fed there was an increased nitrogen excretion during the work and post-work periods of as much as 55 per cent over the preliminary control periods of rest. The increase was greatest on the third or fourth day of fast, then gradually diminished until on about the tenth day a condition was reached in which muscular work produced no increase in nitrogen metabolism. It should be noted that by this time according to the non-protein respiratory quotients obtained by Anderson and Lusk it is probable that the animals had depleted their carbohydrate reserves and were deriving the energy for exercise from body fat. The increase and subsequent decrease in protein metabolism may correspond to the utilization and exhaustion of deposit protein. The energy from the maximum extra nitrogen metabolized during the work and post-work periods amounted to only 3.6 per cent of the total work done.

A preference of the muscle for carbohydrate is shown in the following

experiment. When a well-fed dog was kept for 8 days on a pure carbohydrate diet of 100 to 200 grams of sucrose daily no significant increase in nitrogen excretion during exercise was found. There was a small increase however in some of the post-work periods, notably on the 5th and 6th days.

After following the usual course of nitrogen excretion during fasting and exercise until the 10th day, instead of maintaining a constant level, the extra protein metabolism during work in one experiment increased steadily to the 18th day. As the dog was extremely emaciated it seems probable that this second increase represents a catabolism of tissue protein increasing with the depletion of body fat.

The close agreement in many experiments between the nitrogen excretion during the 3 preliminary periods and the 3 exercise periods, i.e., 49, 47, and 50 mgm. per hour during the former and 48, 50 and 48 during the latter, raises a question as to the validity of a necessary wear and tear protein metabolism for moderate muscular work.

Crystal-fiber diffraction patterns from relaxed and contracted muscles. JANET HOWELL CLARK.

In a previous communication, a theory was proposed to explain the shortening of muscle fibres during contraction which was based on the assumption that the substances in the anisotropic bands pass from the liquid to the solid crystal form when the muscle contracts.

By the use of the monochromatic pinhole method, x-ray diffraction patterns have been obtained with muscles in relaxed and contracted states.

Motor phenomena of the pylorus. L. G. COLE.

A correlation of the anatomical, histological and roentgenological facts and the physiological phenomena of the pylorus. By pylorus we refer to that part of the stomach distal to the corpus or body of the stomach. It includes: The sulcus angularis, the pyloric antrum, the pyloric canal, the pyloric valve and the pyloric cap,—each of which has a motion all its own. These motions combined with others form a still more complex gastric motor phenomena than that previously described by the author.

This paper will be illustrated by synthetic animation, hoping that it may stimulate others to use this method for the dissemination of physiological facts and phenomena.

Determination of the vitamin B requirement of the pigeon and its bearing on the theory of vitamin B function. GEORGE R. COWGILL and B. H. KLOTZ.

Our studies of vitamin B requirement[1] have been extended to include the pigeon. A method was devised for feeding adult pigeons on a diet adequate except with respect to vitamin B (and C).[2] To birds fed in accordance with this technique vitamin B was supplied daily in gelatin capsules in the form of Yeast Vitamine Powder (Harris)[3] in carefully weighed doses varying from 30 to 240 mgm. Twenty birds were used ranging from 300 to 520 grams in weight. The powder used was lot 985, previously tested in experiments on dogs, rats and mice. These quantitative experiments on the pigeon show that the vitamin B requirement of

[1] G. R. Cowgill, A. H. Smith and H. H. Beard: Journ. Biol. Chem., 1925, lxiii, xxiii.
[2] Many investigators have shown that birds do not require vitamin C in the diet.
[3] From the Harris Laboratories, Tuckahoe, New York.

the adult bird is in linear relationship to the 5/3 power of its body weight. This may provisionally be regarded as a function of the product of the body weight and the number of calories metabolized per day. Stated a bit differently the vitamin B requirement appears to be conditioned mainly by the mass of the tissue (weight) and the total metabolism of that mass in unit time (calories per day).

The tentative vitamin formula previously suggested[1] has been simplified. The data from the pigeon, rat and dog agree very well with the simplified expression, whereas those for the mice fit the older formula better. Inasmuch as the error of method was greatest where mice were employed, and simplicity of expression is desirable, the new formula is favored, namely:

$$\text{Vitamin} = K\, W^{5/3}$$

where the precise value of K is peculiar to the species. While such an expression suggests that vitamin B is a structural and metabolic necessity in all cells of the body there are reasons, which cannot be elucidated here, for believing that the data are as yet inconclusive on this point.

The effect of carbon dioxide on the action current of nerve. H. DAVIS, W. PASCUAL and L. H. RICE.

In 1926 Davis and Brunswick suggested that certain increases in the electric response of an excised nerve following a period of moderate activity might be caused by the carbon dioxide liberated by the activity of the nerve. To test this possibility we have studied the relation between electric response and carbon dioxide tension of the surrounding atmosphere, using a gas chamber and string galvanometer as previously described. We find that an excised peroneal nerve of a cat will function for several hours at least surrounded by moist outdoor air (in which the partial pressure of carbon dioxide is 0.2 mm. Hg), and also in an atmosphere freed from carbon dioxide by passage over fused sodium hydroxide.

Increase in carbon dioxide tension causes an increase in electric response up to about 40 per cent at 27 mm. Further increase in carbon dioxide is less favorable, so that at physiological tensions increase in response is negligible, and from 60 to 100 mm. tension the final effect is a depression of 5 to 8 per cent. The new level of response is reached in about half an hour and is maintained for at least an hour as a steady state. The effects are readily reversible. The electric response in control nerve begins to diminish an hour or two after excision under our experimental conditions and falls five to ten per cent an hour during the next two hours. The onset and rate of this fall are not perceptibly affected by carbon dioxide in the concentrations employed, increases or decreases being merely superimposed on it.

Our measurements are made in terms of total quantity of electricity, and our present method does not discriminate clearly between increase in potential and increase in duration of the response; but with carbon dioxide tensions of 20 mm. and over we have definite evidence of a prolongation of the action current. If the increase in electric response is due only to this prolongation, accumulation of carbon dioxide should not materially facilitate conduction. The carbon dioxide produced by the activity of the nerve is presumably quite negligible as a possible cause of "treppe" or "summation in conduction" under physiological conditions.

Further observations on the pressor substance in the blood of certain hypertensives. C. S. DANZER.

The association of hypertension with an abnormal blood in uremia, nephritis, gout and lead poisoning suggests that high blood pressure may be due to some circulating pressor substance in these cases.

To test this possibility whole blood drawn from clinical cases was injected into cats anesthetized with urethane. In preliminary experiments it was observed that the injection of human blood usually depressed the cat's arterial pressure, a condition which was assumed to be anaphylactic. Accordingly the cats were first desensitized by subcutaneous and intravenous injections of small quantities of the human blood to be studied. Shortly thereafter they were injected with 10 to 20 cc. of the same blood and the arterial pressure curve obtained.

In the majority, but not in all, there was a sharp rise of arterial pressure one-half to one minute after the injection of blood drawn from cases of hypertension. The rise was usually of short duration, one to two minutes, but in one animal it lasted two hours. Blood from normal or hypotensive cases failed to produce a pressor effect. These results, although not uniformly positive, as might be expected from the complicated nature of the problem, suggest the presence of a pressor substance in the blood of patients suffering from hypertension.

Blood pressure in the rat. R. R. DURANT.

Using the standard manometric method blood pressure was recorded from the aorta and carotid arteries in 70 rats anesthetized with ether. The pressure in 42 normal adult rats (above 6 months in age) of both sexes was found to vary between a minimum of 92 mm. Hg and a maximum of 150 mm. Hg. In this group the arithmetical mean is slightly below 120 mm. Hg. The mean for males (19 animals) is 123 mm. Hg, for females (23 animals) 116 mm. Hg. The mode for males is above the mean for females and the mode for females is below the mean for males. Between the ages of 4 weeks (50 gram animals) and 6 months (220 gram animals) the average blood pressure rises from 70 mm. to 120 mm. Hg. As shown by blood pressure records the heart and vasomoter system of rats show the usual mammalian reactions to adrenalin and amyl nitrite and to electrical stimulation of the vagus and sensory nerves.

Decrement in nerve conduction. D. J. EDWARDS and MCKEEN CATTELL.

Evidence has recently been published indicating that the nerve impulse conducts with a decrement for a short distance after entering a compressed region. Pressure was obtained by the application of a weight to hard rubber blocks applied to the nerve; a method open to the objection that it involves deformation of the nerve and possibly does not transmit the pressure uniformly to all fibers. For this reason further experiments have been carried out with compression applied through a fluid medium. Our apparatus consisted essentially of two glass cannulae connected by means of a segment of dog's artery through which the nerve (which remained attached to the gastrocnemius of a frog) could be drawn. The cannulae were placed in a chamber of normal saline solution to which pressure was applied from a cylinder of oxygen. Records of the tension developed by the muscle when stimulated on the far side of the compressed area were made at frequent intervals.

A comparison was made of the effect of a pressure of 125 cm. of mercury applied over distances of 8 and 4 mm. of nerve in blocking conduction, as indicated by changes in the tension developed by the muscle. In the first series of experiments, involving 34 observations, the average times required to reduce the tension 50 and 80 per cent with the 8 mm. compression distance were 4.36 and 6.49 minutes, respectively, while the corresponding periods with a 4 mm. compression distance were 6.36 and 10.00 minutes. The figures obtained from preparations from the same frog correspond closely, the deviation from the average in 17 pairs being approximately ½ minute for both the 50 and 80 per cent tension changes.

The results obtained in a second series of observations in which a comparison of the blocking effect of 8 and 4 mm. compression areas was determined successively in the two preparations from the same frog, are similar: In 13 pairs of observations the average figures for the reduction of the tension to 50 and 80 per cent are 4.10 and 6.87 minutes for the 8 mm. distance, as compared to 5.94 and 10.32 minutes for the 4 mm. distance.

Since the method necessarily depends upon a difference of pressure, it is probable that the effects are not uniform throughout the distance involved. Histological evidence indicates a greater forcing out of fluid and compression of the myelin sheath at the limits of the compressed region. This factor is present to an equal degree in both compression distances studied, so that it is safe to conclude that the observed differences in the effectiveness of the given pressure is due to the difference in the length over which it acts. This confirms the earlier work with block compression and indicates conduction with a decrement extending a distance of at least 4 mm. into the unfavorable area.

Cardiac dilatation and hypertrophy. J. A. E. Eyster.

Following experimental lesions in the dog's heart (septal puncture, aortic stenosis, aortic regurgitation) there is an immediate dilatation of the heart. In aortic lesions this dilatation affects mainly the left ventricle in the region of the apex. The dilatation persists for 3 to 10 days and the heart then usually returns to its normal size or is even smaller. If the heart is examined microscopically during the stage of dilatation, acute hydropic degeneration is found (reaction to injury). Subsequent to the period of dilatation the stage of ventricular hypertrophy develops and is complete in 70 to 100 days. If a second lesion is produced a second period of dilatation and hypertrophy occurs as before. If the stage of dilatation is produced by an aortic band and the overload removed by removal of the band on the 3d to 5th day, hypertrophy develops as usual. A continual overload is thus not necessary for the development of hypertrophy. It would thus seem that the hypertrophy following the experimental lesion in the dog's heart is essentially an injury hypertrophy. The factor of continued increased work (work hypertrophy) is thus not essential. Further experiments are in progress to determine whether or not increased work per se plays any rôle in producing hypertrophy of the heart muscle.

The absolutely refractory phase of the alpha, beta and gamma fibers in the sciatic nerve of the frog. Joseph Erlanger, H. S. Gasser and G. H. Bishop.

In two places we have published preliminary data on the duration of the absolutely refractory phases of the alpha, beta and gamma groups of

fibers in the sciatic nerve of the frog. More thorough investigation shows that the published durations in the case of the slower waves, due probably to the brevity of the induction shock (0.06+ sigma) of the coreless Porter coil used as the source of the stimuli, are too long. The method now employed is as follows: A long nerve is used so as to get good separation of the waves. The interval between the first and the second stimuli, the latter from a cored coil, is gradually increased and a record is made of the time separation between them at which there appear in the typical three-waved action potential small delayed alpha, beta and gamma waves, as the case may be. Since the natural maximum amplitudes of the waves decrease in the order given, the amplitudes of the waves traveling in their relatively refractory fibers also have these relative, but much smaller, amplitudes. The accuracy of the end points therefore decreases in the same order. On this account it is possible that the small differences now found between the refractory phases of the alpha, beta and gamma fibers of a nerve, as shown in the table, fall within the experimental error of the method and that the refractory phases of the fibers of a given nerve all are alike.

NUMBER	SCIATIC NERVE OF	ABS. REF. PHASE IN σ			STIMULI PER SECOND	REMARKS
		α	β	γ		
1	Bullfrog (1–2)	1.42	2.06	4.46		
2	Greenfrog 2)	1.20	1.68	3.13		
3	Bullfrog	1.36	1.62	1.94		
4	Bullfrog	0.73	1.06	1.34	13.0	
5				1.20	13.0	Stronger 2nd stim.
6		0.85	0.98	1.17	20.0	Stim. as in 4
7		0.93	1.09	1.26	20.0	Stim. stronger
8		0.91	1.03	1.18	8.3	Stim. as in 7
9	Bullfrog	1.45	1.60	1.49		
10		1.49	1.49	1.49		All appear together
11		1.45	1.45	1.45		All appear together
12		1.68	2.05	2.26		
13		1.77				
14		1.66	2.15			Stronger stim.
15		1.56	2.03–	2.22–		

(1) Erlanger, J., H. S. Gasser and G. H. Bishop. 1924. This Journal, lxx, 624.
(2) Erlanger, J., G. H. Bishop and H. S. Gasser. 1926. Proc. Amer. Physiol. Soc., This Journal, lxxvi, 203.

A study of the effect of varying amounts of oxygen, anoxemia and anesthetic on the sugar metabolism of animal cells. A. M. Estes and W. E. Burge.

The sugars used in this investigation were dextrose, levulose and galactose and the anesthetics ether, chloroform, nitrous oxide and ethylene. The sugar determinations were made according to the method of Benedict. The animal cells used were paramecium caudatum.

Large quantities of paramecia were collected, washed free of debris by centrifugalization and measured in centrifugalizing tubes which were graduated in cubic centimeters. Paramecia sugar preparations were

made with 5 cc. of paramecia in 100 cc. of 0.1 per cent sugar solutions. Oxygen as well as air was bubbled through these 100 cc. preparations in sedimentation glasses at various rates. The organisms were deprived of air and hence of oxygen by introducing 100 cc. preparations into 100 cc. stoppered bottles. Anesthesia was produced by bubbling a mixture of air and the anesthetic in gaseous state through the paramecia sugar preparations.

It was found that the organisms through which small amounts of air and oxygen (5 cc. per minute) as well as large amounts of air and oxygen (400 cc. per minute) metabolized less sugar than did those through which a medium amount of air as well as oxygen (40 cc. per minute) was bubbled. The organisms stoppered in the bottles and hence deprived of oxygen metabolized practically no sugar. Nitrous oxide and ethyline produced no anesthesia and no decrease in sugar metabolism when administered with a liberal amount of air or oxygen while anesthesia and a decrease in sugar metabolism was produced when a small amount of air or oxygen was administered with the anesthetic. Hence ethyline and nitrous oxide produce anesthesia by inducing a state of anoxemia which in turn decreases the sugar metabolism. The decrease in sugar metabolism brought about by excessive oxygen is attributed to the toxic effect of the oxygen on the organisms. Ether and chloroform produce anesthesia and a decrease in sugar metabolism when administered with large as well as small amounts of air or oxygen.

Contributions to the physiology of gastric secretion. I. Gastric phase. JAMES I. FARRELL.

In order to study the gastric phase of gastric secretion, we have made a pouch of the entire stomach. We also cut both vagi in order to prevent any psychic stimulation. A pouch of the entire stomach prevents contamination of the gastric secretion by regurgitated material from the intestine, and saliva from the mouth.

Through a fistula in the anterior abdominal wall we have placed various food substances into the stomach. By analyzing the substance before and after introduction, and following the secretion for several hours after, we have been able to note the effect on the gastric secretion. Care was taken not to introduce large amounts, since such a pouch can be stimulated mechanically.

Distilled water, tenth normal HCl, sodium bicarbonate, all gave no change in the secretion.

When gastric juice of a high acidity was placed in the stomach, it did not increase the secretion; on the contrary the acidity of the gastric juice introduced was lowered.

Olive oil definitely inhibits gastric secretion.

Alcohol stimulates gastric secretion, solutions of high percentage produce a secretion of high acidity and large amount, weaker solutions correspondingly produce less secretion of a lower acidity. The so-called "bitters" of which tincture of gentian is a representative, stimulate the flow of gastric secretion. Water solutions of gentian did not influence the secretion. We are of the opinion that the alcohol is the stimulating factor in the tincture of gentian, and not the gentian itself.

Raw meat juice stimulates the flow of gastric juice; when however the meat juice has been boiled for one-half hour, it loses its power to stimulate

the gastric secretion. The products of meat digested with gastric juice also stimulate gastric secretion. Liebig's meat extract is also capable of stimulating gastric secretion.

Lactic acid, Armour's scale pepsin, NaCl, bread juice, sucrose did not stimulate gastric secretion.

The work is still in progress and a large variety of foods, and physiologically important substances are being tested.

The increased gas exchange of frog nerve during activity. W. O. FENN.

A microrespirometer has been designed sensitive enough to give a deflection of the kerosene index drop of 0.1 mm. for an oxygen consumption of 1.4×10^{-5} cc. Frog nerves stimulated electrically in this apparatus with 200 shocks per second consume an increased amount of oxygen over and above their resting rate. At a stimulation frequency of 200 per second the excess oxygen is 0.315 cu.mm. per gram of nerve per minute of stimulation; at 100 per second it is only slightly less, 0.268 cu.mm. per gram per minute. Doubling the frequency of stimulation over this range increases the oxygen consumption only 1.18 times. The negative variation under similar conditions is increased approximately in the same ratio, i.e., 1.15 times. Calculation shows that in the small frog nerve the oxygen supply is adequate. When the nerves are stimulated in the absence of sodium hydroxide to absorb the carbon dioxide the apparent R.Q. is less than 1. A method has been worked out for measuring the carbon dioxide dissociation curve of nerve tissue by means of which the apparent respiratory quotients can be corrected. That the true respiratory quotient of the excess metabolism of nerves is very nearly 1 can be shown by stimulating the nerves in the absence of sodium hydroxide and in an atmosphere of 10 per cent carbon dioxide. Under such conditions the correction for absorbed carbon dioxide by the buffers of the nerve is very small. Indications are that stimulation is followed by an initial extra consumption of oxygen which is followed by a compensatory delayed outburst of carbon dioxide so that the net change is relatively small.

The frequency of motor nerve impulses in the sustained flexion reflex. ALEXANDER FORBES, ANTONIO BARBEAU and LUCINDA H. RICE.

Some physiologists contend that the apparent rhythm of the electromyogram in sustained reflexes reveals directly the actual frequency of motor nerve impulses; others have held that the nerve impulse frequency is higher than that which appears in the electromyogram. Cooper and Adrian (1924) inferred from certain experiments involving changes of temperature that in the case of the flexion reflex in the spinal cat the motor nerve-impulse frequency could be detected in the electromyogram and was in the neighborhood of 250 per second. Forbes and Olmsted (1925) by the alcohol block method showed that in the crossed extension reflex in the decerebrate cat the majority of motor nerve impulses involved in maintaining contraction were subnormal and therefore occurred during the relatively refractory period; from this it was inferred that the dominant frequency was more than 300 per second. They also pointed out that the conclusion of Cooper and Adrian did not necessarily follow from their observations. It was left an open question whether the nerve impulse frequency in the case of the sustained flexion reflex was much lower than in the crossed extension reflex.

We have applied the alcohol block method to the sustained flexion reflex in the case of the decerebrate cat, both before and after low spinal transection. The flexion reflex usually starts with a volley of full-sized impulses, after which the differential blocking of reflex impulses by the narcotic shows their dominant frequency to be usually above 300 per second and often above 400 per second.

COOPER AND ADRIAN. 1924. Journ. Physiol., lix, 61.
FORBES AND OLMSTED. 1925. This Journal, lxxiii, 17.

Chronaxy of smooth muscle. HENRI FREDERICQ and MARCEL FLORKIN.

So far, only a few papers have been published on the excitability of smooth muscle. It seemed necessary to us to ascertain the experimental conditions affecting this excitability. The experiments have been performed either on the cloaca of the frog or on thin strips cut from the bladder of the rat.

The estimation of the excitability has been made by the chronaximetric method by means of constant make currents. One of the electrodes is a thin thread of silver, covered with a layer of silver chlorid. The other electrode is a liquid one (bath of Ringer's). The size of the active electrode does not seem to affect the value of the chronaxy.

In the frog's cloaca, at the end of the winter, the chronaxy is in rough figures, ten times longer (average, 500) than at the end of summer (average, 50).

Stretching the muscle by weights of 10 to 30 grams shortens the chronaxy of smooth muscle just as it does in striated muscle and in the turtle's heart. An increase of the osmotic pressure (addition of saccharose 5 per cent) of the Ringer's bath lengthens the chronaxy. This seems to be a consequence of the decrease of the imbibition of the tissue. The chronaxy is also lengthened and the tonus is diminished by addition of amino-acids (glycocol 0.5 per cent; d-alanine 0.5 per cent) to the Ringer's bath. In no case were the variations of the rheobasis (galvanic threshold) systematic.

The relation of the shape of the action potential of nerve to conduction velocity. H. S. GASSER.

In the elaboration of the membrane theory, particularly as done by Lillie (1), the velocity of conduction in a tissue is associated with a corresponding duration of the rising phase of the potential change. In a nerve trunk, however, the duration of the rising phase is constant, within the limits of error, for fibers of all velocities. Here the velocity is shown by Gasser and Erlanger (2) to be dependent upon another variable, the diameter, with which it varies directly.

To obtain more suitable data on the rising phase, experiments were performed in which some of the errors present in the older observations, cited in support of the association of the rising phase and velocity, were eliminated. Leads were made from sheathless dog-phrenic nerves at the stimulating cathode, to eliminate the conduction error and a large artefact. The duration of the rising phase was caused to vary by temperature because this has much less differential action than, for example, drugs or pressure. The potential wave of the nerve is then fairly representative of the wave in an axon.

The results are uniformly consistent; they show that the durations of

the rising phases are indeed inversely proportional to the velocity ratios in the fastest fibers. Otherwise stated, the crest is a constant distance behind the front at all velocities. Other properties as maximum slopes of the wave front, the heights, and the areas did not show any simple relation to velocity. The relationship between velocity, rising phase, and diameter may be stated empirically with the equation: velocity equals a constant times the ratio of the diameter to the duration of the rising phase.

The relationship holds in spite of the fact that the axon potential was found to fall in the colder fibers. A theoretical reconciliation can, however, be made between this observation and the bioelectric circuit theory. The behavior of the changes in the potential form at different velocities can, therefore, be interpreted as compatible with such a theory. It does not exclude other possibilities.

(1) LILLIE: This Journal, 1914, xxxiv, 414.
(2) GASSER AND ERLANGER: This Journal, 1927. In press.

The adrenal mechanism and the modification of insulin action by post-pituitary extracts. E. M. K. GEILING and S. W. BRITTON.

Protection against insulin hypoglycemia and convulsions in cats and dogs is shown definitely to be afforded by the simultaneous administration with insulin of extracts of the posterior pituitary lobe. Very large doses of the extract are necessary to produce this effect. If smaller amounts which nevertheless bring about extreme circulatory disturbance are employed, convulsions are merely postponed. The action of superconvulsive doses of insulin (4 to 6 units per kilo body weight) is not counteracted by very large amounts (3 cc. to 4 cc.) of pituitary extract (U. S. P.). If the adrenal medulla is inactivated by removal of the right and denervation of the left gland, the protection against insulin by pituitary extract (given simultaneously) is very markedly lessened. When it is administered in such cases during hypoglycemia, severe convulsions or all signs of muscular weakness may nevertheless be abolished and the blood sugar level may be sharply raised. Animals with the adrenal glands intact may be similarly restored, although the blood sugar percentage often remains only slightly or not at all changed near the convulsive level. Pituitary extract may therefore completely offset all symptoms of insulin shock without appreciably altering the glycemia in the general circulation. In contrast with the foregoing is the observation that pituitary extract has no apparent constant effect on the blood sugar level in the fasting normal or adrenal inactivated animal. The depression of sympathetic nervous activity by ergotamine does not affect the restorative potency of pituitary extract given during the hypoglycemic reaction. When pituitary extract is given simultaneously with insulin to either normal or medulliadrenal inactive animals, and the blood sugar has later fallen to a low level, adrenalin may abolish the apparent general reaction (weakness or convulsions), although the hypoglycemia commonly remains unaffected. The evidence establishes that there is present in pituitary extract a protective factor against insulin hypoglycemia and convulsions, and that facilitation of the action of the extract is afforded by the presence and augmented secretion of the medulla of the adrenal glands.

The influence of the vagi upon intra-auricular block. Electrographic studies. WALTER E. GARREY and RICHARD ASHMAN.

Studies on intra-auricular block reported by one of us (G) have been

continued by electrographic methods. Previously obtained tracings from turtle hearts had shown that vagus stimulation usually increased the degree of block but that in certain instances the block disappeared or was reduced; sometimes combinations of these two effects might be obtained on the heart as a result of a single stimulation of the vagus. Lewis and also Drury had reported that block by compression or cooling always disappeared upon vagus stimulation. In the present series on the turtle, Chrysemys elegans, only the opposite result was obtained: invariably there was an increase in the degree of block.

Electrograms were obtained from the two auricles in situ, the ventricle having been removed. The lateral third or fourth of one auricle was nearly separated from the rest by incision, excepting for a broad attachment at the apex. The heart rate was rigidly controlled by induction shocks applied to this semi-detached slip, and at the same time stimulation of vagus fibers in the rest of the auricle, from which the action current was led, was avoided. It was thus possible to use strong vagus stimulation without chronotropic effects. Mild compression was applied by a clamp across the base of the opposite auricle.

There is no ambiguity in the electrograms. In every case faradic stimulation of the neck vagus, depending upon its strength, caused either partial or complete A-A block. No attempt was made to differentiate the effectiveness of right and left vagi; both were effective but not always to the same degree. The inotropic effects, as observed, were typical. Stimulation of the intra-cranial vagus, tried on one animal after cutting the cervical cord, yielded the same results.

Our method made it possible to show further that mild vagus stimulation may very markedly prolong A-A conduction time without actual block; that stonger stimulation still further increases that interval until block appears; and that when block does occur, the longer rest periods of part of the conducting pathway may shorten conduction time to less than its maximum for such impulses as are conducted. The few curves obtained illustrating recovery of A-A conduction during mild vagus stimulation were similar in their main features to those obtained by Lewis and Master for A-V conduction in the mammal.

Monophasic action potentials of tortoise ventricular strips. Recorded from the site of stimulation. ARTHUR S. GILSON, JR.

The electrical change occurring at a point in cardiac tissue during a single heart cycle is a prolonged state of negativity which commences with the excitation of the tissue and lasts through the period of contraction. Such a monophasic action potential record from a localised region is seldom if ever recorded directly because of the diphasic effects from the two lead-off electrodes when diffuse poorly localised leads are not used.

Action potentials of strips of tortoise ventricular muscle were amplified by a one panel, thermionic vacuum tube amplifier and recorded by use of a capillary electrometer. One of the two lead-off electrodes was made the cathodal electrode of the stimulating break shocks from an induction coil. The action potential recorded was, therefore, led from the site of stimulation and from some other point on the strip. All records which were obtained showed some diphasicity.

A purely diphasic record obtained from a strip with the two electrodes placed on tissue in similar states shows an R-wave rising immediately out

of the record of the shock, a drop back to the base line, an interval during which the curve nearly follows the base line, a T-wave of sign opposite that of the R-wave, and a slow final return to the base line. Severe fatigue or injury of the tissue at one electrode causes predominance of the action potential effective at the other electrode, but never a completely monophasic condition.

Diphasic records as described above are due to a series of monophasic action potentials at a series of points along the strip in which the limiting curve of the potential of the excited tissue with respect to resting tissue rises quickly after stimulation. This negativity is continued as a plateau which is maintained through the period of contraction. The electrical negativity then falls off, rapidly at first, more slowly as it approaches the base line. In fatigued or injured tissue, the plateau is maintained for a much shorter time. The usual diphasic curve may be considered as the expression of the algebraic sum of two such monophasic action potentials at the lead-off electrodes plus a certain amount of modification due to extrinsic effects at other parts of the strip.

The R- and T-waves as recorded from such preparations are, therefore, not an expression of two distinct processes separated by a definite time interval, but are the expression of a series of prolonged processes occurring along the strip as the transmitted impulse excites each point in turn, the region last excited being the last to return to a resting state and therefore showing a final negativity or T-wave. The time of the rising phase of the R-wave varies, when other conditions are constant, with the time required for the spread of the impulse from the proximal to the distal electrode. A similar interpretation may be given to the electrogram from auricular tissue.

The effect of insulin injected directly into the renal artery upon the nitrogen and sugar elimination of the phlorhizinized dog. JACOB GOLDSTEIN and DORAN J. STEPHENS.

Dogs were phlorhizinized for three days. Under amytal anesthesia the ureters were cannulized and 1½ units of insulin in Locke's solution per kilo of body weight injected slowly into the right renal artery. There is a decrease in the sugar elimination and an increase in the nitrogen elimination of the injected kidney. The uninjected kidney does not show any marked change until some time later when the insulin has had time to reach it in sufficient concentration. Control experiments were run in which Locke's solution alone was injected. The effects on sugar and nitrogen excretion seem to be due to a genuine antagonistic action of the insulin to phlorhizin.

Studies in the metabolism of the bile acids. CARL H. GREENE and MARTHA ALDRICH.

The studies of Stadelmann, Whipple and Schmidt and their associates on the metabolism of the bile acids are well known. Because of the limitations of the methods used their studies were confined to the changes in the bile and urine. We present a quantitative adaptation of the Pettenkofer reaction by which glycocholic acid in amounts of from 0.10 to 0.50 mgm. in a volume of 8 cc. can be determined with an error of approximately 5 per cent. This reaction has been applied to the alcoholic extract of 5 cc. of blood and the method of extraction selected to permit the determination of maximal amounts of the Pettenkofer reacting material. Normal

blood gives a color equivalent to that from 3 to 6 mgm. of glycocholic acid per 100 cc. while bile acids added to blood may be recovered with an average loss of but 0.5 mgm. per 100 cc. In general the recovery is greater than 90 per cent.

The method can be applied to bile and its accuracy has been tested by comparison with that of Schmidt and Dart. The values obtained for the analysis of bile by the Pettenkofer method agree well with those obtained by the gasometric determination of the amino-nitrogen liberated by alkaline hydrolysis. When the use of small amounts of material, or the ease and rapidity of analysis enter into the choice, this modification of the Pettenkofer reaction would seem to be the preferable method.

When whole bile or bile salt preparations are injected intravenously the bile acid content of the blood increases markedly, depending on the dose given and the speed of the injection. After such an injection the bile acids leave the blood so rapidly that even with maximal doses the excess is removed within two hours.

Because of the small amounts required for analysis, it has been possible to follow more closely than heretofore the excretion of bile acids in the bile of a dog with a biliary fistula. The curve of excretion has been studied and the rapidity of the excretion of bile acids and the independence of the bile acids and bilirubin is reëmphasized.

When bile or bile acids are given by mouth, the amount in the blood of the portal vein is increased though the level in the systemic circulation (jugular vein) is unchanged. This speaks for the entero-hepatic circulation of the bile acids.

Delay of blood in passing through the lungs as an obstacle to the determination of the CO_2 tension of the mixed venous blood. W. F. Hamilton, J. W. Moore and J. M. Kinsman.

A mixture of CO_2 and O_2 was rebreathed (according to the Henderson-Prince technique as modified by Bock and others) until the CO_2 tension was constant. The result varied directly with the time allowed for rebreathing. If the excess CO_2 of repeatedly rebreathed air over the normal alveolar air at the end of fifteen seconds is taken as 100 per cent, at the end of six seconds it is 74 $\pm$ 1.5 per cent, at the end of nine seconds it is 82 $\pm$ 1.4, at the end of twelve seconds, 87 $\pm$ 0.83 per cent, at the end of twenty-four seconds it is 117 $\pm$ 1.5 per cent.

This same progressive increase holds if enough CO_2 is introduced between rebreathings to counteract the lowering of the CO_2 percentage which results from dilution by the residual (alveolar) air. This prevents all outward diffusion and shows that the progressive increase is not the effect of lagging diffusion of CO_2.

To show conclusively that arterialized blood in the lungs is responsible for the low tensions in the shorter experiments, the CO_2 content of a mixture of the normal residual air, and a known amount of "virtual venous" air were carefully calculated (238 cc. average of eight experiments). The virtual venous air was rebreathed for four and eight seconds, and its CO_2 content then determined. At the end of four seconds, 13 cc. of CO_2 had disappeared, and at the end of eight seconds, 1 cc. was still missing. Thus all of the CO_2 which diffuses from the venous blood entering the lungs, as well as a part of that which has been inspired in the "virtual venous air" leaves the lung air.

These findings are explained on the assumption that when one starts to re-breathe vitrual venous air, the blood in the lungs is venous only in part, and arterial elsewhere. The tension of the re-breathed air is an expression of the average of these various blood tensions, an average which gradually changes as the arterial blood leaves the lungs and as the venous blood enters, and is further changed as re-circulated blood begins to come back to the lungs.

If we calculate the cardiac output upon a basis of the "venous" tension at the end of twenty-four seconds, the figure comes out at least twenty per cent lower than if we calculate it on the basis of the "venous" tension at the end of eight seconds. The "venous" tension varies continuously between these values.

These results, if substantiated, cast serious doubt upon all methods of calculating the circulation based upon the use of the lungs as an aerotonometer for CO_2. The same difficulties would apply to oxygen, but might not apply to nitrogen.

Effects of epinephrin and of sympathetic stimulation upon skeletal muscle. F. A. HARTMAN, J. I. EVANS and H. G. WALKER.

It is possible to observe the skeletal muscle of the cat under the high power of the microscope by transmitting light to the muscle through a glass rod. In this way individual muscle fibers and the capillary circulation may be studied, provided a thin muscle is used. So far the sartorius muscle has proven the most satisfactory.

Epinephrin in small amounts (0.4 cc., 1:100,000) causes new capillaries to open and capillaries already open to dilate. Venules appear to react in a similar fashion. A striking change appears in the muscle tissue itself. First, it becomes more translucent. Then with larger doses this effect not only becomes more marked but the muscle fibers stand out more clearly and the cross striations which may not have been visible before are easily seen. The muscle fibers also begin to twitch transversely.

With the larger doses, the dilatation of capillaries may increase to a certain point beyond which constriction begins to appear. In very young kittens epinephrin even with small doses causes constriction of the capillaries instead of dilatation.

If the nerve is cut to a muscle and time is allowed for degeneration (in adult cats) epinephrin even in large doses causes only dilatation of the capillaries, venules and arterioles. But with the larger doses the veins and arteries become constricted.

Stimulation of the sympathetic chain in the lumbar region by rapidly repeated induction shocks causes only dilatation of the capillaries and venules. We have not observed constriction from such stimulation however the rate or intensity of the shocks were varied. Very weak stimulation makes the field more opaque. As the strength of stimulation increases a point is very soon reached which causes the field to become much brighter. This is accompanied or sometimes preceded by very fine and extremely rapid vibration of the muscle fibers. The vibration soon changes to twitching resembling that produced by epinephrin.

The twitching produced by chemical stimulation, as drying, is entirely different from that resulting from epinephrin or sympathetic stimulation.

Seasonal periodicity in man. Part 1. Basal metabolism, respiration, cardio-vascular condition, blood-gas capacity and cell count. FRED. R. GRIFFITH, JR., GEO. W. PUCHER, KATHERINE A. BROWNELL, MABEL E. CARMER and JEANNIE D. KLEIN.

Two normal men were under observation for two years and three normal women for one year. We present what is the apparent course of curves plotted from the monthly averages of the two-year period.

1. Oxygen consumption follows a very regular curve which is lowest late in the summer. 2. CO_2 production varies within wider limits than oxygen and fails to show a definite seasonal rhythm. 3. The respiratory quotient is thus highest in the summer. 4. The energy expenditure, calculated as calories per square meter per hour, is lowest in summer. 5. Respiratory rate is greatest in summer, associated with a marked shortening of the expiratory phase. 6. The minute volume is lowest in late spring and highest in the fall. 7. The tidal volume is lowest in the early part of the year. 8. The composition of the expired air depends entirely on the minute volume. 9. Alveolar air showed no seasonal change but was related to menstrual cycle; *i.e.*, the CO_2 tension was lowest just before and highest mid-way between periods. 10. The basal pulse-rate follows a regular curve which is lowest in summer. 11. Systolic pressure (reclining and standing) is lowest in the spring. 12. Blood gas capacity (for both O_2 and CO_2) was highest in spring and fall and lowest in winter. 13. Blood counts show the reds highest in the summer, the whites highest in the spring. Differential counts show the small and large mononuclears, eosinophiles and basophiles lowest during the summer while the percentage of the polymorphonuclears and transitionals increases at this time.

Refractory period in heart. C. C. GUTHRIE.

Observations on terrapin and frog hearts abundantly confirm the conclusions of others who have found the absolute refractory period to comprise the latent and contraction phases. The widely quoted figure of Marey is misleading in that the two premature ventricular responses following stimulation during ventricular contraction are due to premature impulses of extra ventricular origin, premature auricular contraction invariably preceding such ventricular response. That this occurred in Marey's experiments is evidenced by the latent periods, time of premature contractions, and change of rhythm as shown in his tracings.

The use of stimuli of unphysiological strength, i.e., stronger than are necessary to elicit direct premature response when applied in the beginning of relaxation, is deceptive, as the irritation set up may be prolonged beyond the end of the absolute refractory period. In terrapin, the absolute refractory period is shortest in sinus, and longest in ventricle.

No evidence of a supernormal phase of excitability has been obtained either in spontaneously contracting tissue or in tissue contracting in response to rhythmic impulses.

The latent period is a concrete physiological phenomenon, subject to conditions that affect other heart phenomena and may show enormous variations. To consider absolute refractory period or conduction phenomena intelligently, it must be taken into account. In some experiments apparently showing lengthening of conduction time, i.e., lengthening of the A-V interval, a great part of this was due to extension of ventricular latent period.

Marey's method for measuring the refractory period, with due control of current strength and spread, has proven the most accurate. The method based on the rate of stimulation at which response fails, introduces unknown or uncontrolled factors, e.g., under certain conditions summation effects or the opposite,—depression. (This Journal, 1917, xlii, 598.) Even a single stimulus in absolute refractory period may demonstrably extend the period (loc. cit.), or as Carlson in 1906 showed, at beginning of relative refractory period, a stimulus may elicit multiple response. In character, the multiple stimulation method is more of a resistance or endurance test.

Vagus extension of refractory period is most pronounced in auricles, as shown by failure of earliest premature response to a threshold stimulus, and by coördinated rhythmic sino-ventricular contraction during auricular stand-still from stimulation of a vagus nerve trunk, or induced from a single stimulus applied to auricles.

The vagal reflex control of the respiratory centre. Peripheral mechanical and peripheral chemical factors. ALRICK B. HERTZMAN and ROBERT GESELL.

The relative significance of peripheral mechanical and peripheral chemical factors in the vagal reflex control of the respiratory centre was studied in the dog by anatomically and functionally isolating the head, except for the vagi, from the trunk by crushing through the neck with a powerful vise. The head was kept alive by intercalating it in the carotid-jugular circulation of a second animal. The activity of the respiratory centre of the isolated head was followed by recording the movements of the cricoid cartilage. The trunk was kept alive by artificial ventilation.

In such a preparation, mechanical factors, such as changes in the state of tension of the lungs, appeared important in eliciting vagal respiratory reflex effects. Suspension of artificial ventilation of the isolated trunk elicited an immediate increase in the "respiration" of the head. Changes in depth of ventilation had similar immediate effects.

Reflex stimulation of the respiratory centre of the isolated head was frequently, yet inconstantly, obtained by administration of carbon dioxide to the trunk and in several cases by the injection of sodium cyanide into the trunk. The injection of sodium carbonate and of sodium bicarbonate into the trunk exerted no reflex influence on the "respiration" of the isolated head. This is in contrast to the constancy of effects of similar administrations to the donor.

Further observations on changes in acidity of the circulating blood and cerebro-spinal fluid in relation to respiratory control. ROBERT GESELL and ALRICK B. HERTZMAN.

Observations on changes in acidity of arterial and venous blood and cerebro-spinal fluid with the use of the manganese dioxide, the quinhydrone and the hydrogen electrodes have been continued.

As was previouly shown, mechanical asphyxia with the lungs filled with oxygen produced a greater increase in acidity of the arterial blood than asphyxia of similar duration with the lungs filled with room air. It is now found that the cerebro-spinal fluid may turn more acid with mechanical asphyxia when the lungs are filled with room air. The view of the lack of correspondence between changes in blood and tissue acidity is thus supported.

Temporary hemorrhage which increases the alkalinity of the arterial blood and the acidity of the venous blood may increase the acidity of the cerebro-spinal fluid.

Administration of air poor in oxygen may simultaneously increase the alkalinity of the arterial and venous blood and of the cerebro-spinal fluid. With prolonged administration increased alkalinity frequently gives way to increased acidity of the blood and cerebro-spinal fluid. The directional changes in acidity were more constant in the arterial blood than in the venous blood and cerebro-spinal fluid.

Intravenous injection of sodium cyanide increased the alkalinity of the arterial and venous blood. During recovery the blood frequently turned distinctly acid. The initial alkaline effects of cyanide may give way to increased acidity during constant artificial ventilation. This agrees with the greater alkaline effects of hemorrhage and low oxygen during normally augmented ventilation.

The experiments support the view that cells commonly turn acid as the blood turns alkaline and alkaline as the blood turns acid. The probability of the occurrence of increased cellular acidity with mechanical asphyxia, hemorrhage, low oxygen and cyanide agrees with the importance of cellular acidity in respiratory control. The frequent inhibition of pulmonary ventilation with increasing acidity of the blood and the frequent inverse relation between blood acidity and pulmonary ventilation is further upheld.

Disagreement between the manganese dioxide electrode and the other electrodes with the greater disturbances in oxidation (particularly marked with the administration of cyanide) is of a nature to indicate a liberation of reducing substances by the tissues into the blood and body fluids.

The respiratory quotient of exercising muscle. HAROLD E. HIMWICH and MILTON I. ROSE.

The respiratory quotients of exercising muscles of dogs have been determined by analyzing simultaneous samples of arterial and venous blood for O_2 and CO_2 by the method of Van Slyke and Neill. The drawing of the blood occurred at various times from the third to the fifteenth minute of exercise. For comparison the respiratory quotients of the whole animal while at rest were estimated by analysis of the expired air. Forty-nine experiments were performed on thirty dogs. The experiments fall into two major groups—one on dogs starved from five to fifteen days and another on fed dogs. Each of these groups may again be divided into two minor groups—some in which the venous blood was drawn directly from the femoral vein, carrying the return of the whole lower extremity, and others where the venous blood was drawn after the circulation of the gastrocnemius muscle and the flexor digitorum sublimis had been isolated. The results are as follows:

NUMBER OF EXPERIMENTS	CONDITION OF DOGS	PREPARATION	AIR R.Q. RESTING ANIMAL	BLOOD R.Q. EXERCISING MUSCLE
16	Starved	Lower extremity	0.81±0.03	0.81±0.05
11	Starved	Isolated muscle	0.80±0.03	0.78±0.06
8	Fed	Lower extremity	0.90±0.02	0.91±0.04
14	Fed	Isolated muscle	0.94±0.02	0.98±0.07

Conclusion: Respiratory quotients of exercising muscle obtained by analysis of blood drawn after the third to the fifteenth minute of exercise are close to those of the entire animal at rest obtained from expired air. The respiratory quotient of exercising muscle of starved dogs is close to 0.80, and that of fed dogs is 0.90 or above.

The total energy requirement of the white rat for growth and activity. F. A. HITCHCOCK.

A study has been made of the total food consumed by (1) rats during the first ninety days of their life, and (2) by adult rats kept in activity cages. During the first thirty days the total food consumed by the mother and her young was measured. A control group of eleven litters (81 young) were fed a ration of whole wheat flour, casein, and cotton-seed oil. The average increase in weight of the young was 40 grams (700 per cent) and the food consumed was equal to 11.5 Calories per gram increase in weight. A second group of 14 litters (100 young) were given all the fresh meat they would eat in addition to the ration the control group received. The average increase in weight of this group was 55 grams (900 per cent) and the food consumption was equal to 10.6 C. per gram increase in weight.

At the age of thirty days the young were weaned, the sexes separated, and the experiment continued. The results show that growth is accomplished most economically when the per cent increase in weight is the greatest. Since the meat-fed rats grow faster, they also grow more economically. From 30 to 60 days the controls gained 126 per cent in weight at a cost of 11.5 Calories per gram increase. The meat-fed rats gained 174 per cent at a cost of 10.8 Calories per gram increase. From 60 to 90 days the controls gained 32 per cent in weight at a cost of 26.1 Calories per gram increase, while the meat fed animals gained 30 per cent at a cost of 29.3 Calories per gram increase in weight. If the per cent increase in weight is plotted against the cost in Calories of gram increase, the curve obtained is a rectangular hyperbola represented by the expression $Y = \frac{K}{\sqrt{X}}$ where Y is Calories per gram increase in weight and X is the per cent increase in weight. The constant K is dependent upon age.

The results obtained on the energy cost of activity are extremely variable, but the averages show that the daily food intake is increased about 2 Calories per kilo of body weight for every thousand meters run by the rats. This figure does not seem to be affected by the amount of protein in the diet.

Toxicity of bile. O. H. HORRALL.

The study of the toxicity of bile was undertaken because of the prevalence in the literature of the statement that bile itself is practically non-toxic.

The following results were obtained:

1. Bile is toxic when injected intraperitoneally, subcutaneously and intravenously.
2. When bile is injected in sufficient quantity to cause death in twenty-four hours the presence of bacteria does not modify its toxicity.
3. The toxicity of bile is not modified by sterilizing or freezing.
4. Bilibrubin is non-toxic, and so far as I can determine, is inert.

5. Dialyzed bile has the same toxicity as bile.
6. Non-dialyzed portion of bile is non-toxic. (This eliminates the possibilities of proteins being the toxic agents.)
7. Pure bile acids are non-toxic. (Very slightly soluble.)
8. The salts of bile acids, such as sodium glycocholate and sodium taurocholate, are toxic.
9. The only toxic agents in bile are the salts of bile acids.

Further attempts to experimentally increase the hormone output by the thyroid gland. L. HEKTOEN, A. J. CARLSON and R. SCHULHOF.

In dogs under barbital anesthesia and with the cervical sympathetic nerves severed, there is considerable spontaneous variation in the concentration of thyroglobulin in the thyroid vein blood as determined by the precipitin method. These variations appear not only in different dogs, but in the blood from the two thyroid lobes of the same dog, and in the blood from the same lobe during the course of a single experiment. In experiments on nine dogs the sympathetic nerve stimulation, direct thyroid massage, and intravenous adrenalin and pilocarpine failed to increase the thyroglobulin in the thyroid vein blood above that of the variations in the controls. In one experiment stimulation of the nerves to the thyroid was followed by an increase in the thyroglobulin concentration greater than in the controls. This may be due either to increased rate of thyroid secretion or to decreased blood flow through the gland.

Observations upon the knee jerk in "high" and "low" spinal preparations. P. M. HARMON and J. F. FULTON.

Examination of the knee jerk by the method of Ballif, Fulton and Liddell (Proc. Roy. Soc., 1925, xcviii B, 589), has shown that the susceptibility to inhibition, and the duration of the period of complete inhibition evoked by single break-shock stimuli tend to be greater in low spinal (*e.g.*, 1st lumbar) preparations than in high (*e.g.*, 5th cervical). This precludes the possibility that the prolonged period of "inhibitory after-discharge" in spinal preparations (1 to 2 seconds) is due to the time spent in traversing the so-called spinal delay paths, and it suggests, in accordance with the theory of Sherrington (Proc. Roy. Soc., 1925, xcvii B, 519), that inhibition is due to the liberation centrally of depressant qualities. The knee jerk of spinal preparations is a tetanus, and it tends to be of greater duration in the "low" spinal animal than in the "high".

The effect of experimental pyloric stenosis on gastric secretion. A. C. IVY, E. H. DROEGEMUELLER and J. MEYER.

An experimental pyloric obstruction was made in twelve Pawlow pouch dogs. Seven of the dogs survived for a period of from three and one-half to seven and one-half months. The stenosis first caused a decrease in secretion, and later four of the seven showed a hypernormal secretion, one a normal secretion, and two a decreased secretion. The last two animals should probably be ruled out of the series because their pouches did not reflect the secretory activity of the stomach. The cause of the hypernormal secretion was discussed.

It should be emphasised that before Pawlow pouches are used in an experimental study it must be shown that they reflect the secretory activity of the stomach.

A marked hypertrophy of the stomach occurred in four of the seven dogs.

Adrenal auto-transplantation. A. C. IVY and E. OLDBERG.

A method has been evolved by which a functioning adrenal has been transplanted with part of its original blood supply running to it by means of a pedicle, into the omentum. The left adrenal is first mobilized and surrounded by a cape of omentum, the adrenal vein and all other vessels than those entering the inferior pole, being ligated. At a subsequent operation, the right adrenal is removed. Such dogs have lived in apparent health for a period of two weeks, at which time they were subjected to further operative procedure. Other animals have at the time of this writing, been living in evident good health for sixteen days on such a pedicled transplant, with the right adrenal absent. The "transplant" itself has been present over six weeks in the last mentioned animals.

Histologic studies of the transplants in six of our animals, show that at least some anatomically normal tissue may be expected. Other areas show varying degrees of degeneration which seems to be selective with respect to the medulla. The vessels contain blood and there is some evidence of formation of new blood supply from the omentum.

We propose to ascertain the viability of the transplanted gland with its pedicle cut, after a reasonable length of time has elapsed during which a new blood supply can be developed.

Does methylene blue enter living cells? MARIAN IRWIN.

A spectrophotometric determination shows that the dye which enters from methylene blue solution into the vacuole of living cells of the marine alga, *Valonia macrophysa*, is not methylene blue but its less basic, lower homologue, trimethyl thionin, which is present in the methylene blue solution. The cell wall of living cells is capable of absorbing both methylene blue and trimethyl thionin.

The rate of hemolysis in strongly hypotonic solutions and its relation to the question of the osmotic properties of cells. M. H. JACOBS.

Because of its rapidity, the rate of hemolysis in strongly hypotonic solutions has been little studied, though it is of considerable interest in connection with the problem of the exchange of water between cells and their surroundings. In the present investigation a simple apparatus for standardizing the turbidity of suspensions of erythrocytes has been used to study the rate of hemolysis where the times involved exceed one or two seconds, as is usually the case with human erythrocytes. Where the process is completed in a fraction of a second as is frequently the case with the erythrocytes of the sheep, a modification of the apparatus devised by Hartridge and Roughton for the study of hemoglobin reactions has yielded satisfactory results.

The rate of osmotic hemolysis depends chiefly on that of water intake by the erythrocytes which in turn is determined at any given time, a, by the distance of the system from osmotic equilibrium and b, by a fundamental velocity constant characteristic of the process itself under the given conditions. A change in conditions may affect the observed rate in an apparently complicated way by producing opposite effects on the two factors in question. Thus, a change in the reaction of the hemolytic solution in the alkaline direction increases, and one in the acid direction decreases the value of the fundamental constant. But in each case the effect on the point of osmotic equilibrium is such as to act in the opposite sense, and

the net result is an acceleration of the process at the lowest concentrations and a retardation at higher ones by alkaline solutions, with the reverse condition in the case of acid ones. A mathematical analysis of the results obtained has been attempted.

Studies on the knee jerk. CARL A. JOHNSON.

The author described a knee jerk apparatus, simple in construction and dependable in operation in which an electromagnetic hammer strikes the patellar ligament, not only at any desired time interval, but also with any degree of force. The apparatus is adapted for use on man and lower animals. It has been in uninterrupted operation for 17 hours.

After a study of various cord depressants (paraldehyde, barbital, ether inhalation), and excitants (strychnine), the author studied particularly the effect of morphine on the cord in anesthetised and unanesthetised animals (spinal animals) following the injection of moderate doses of this drug (15 to 120 mgm.). Possible inhibitory effects on the cord obtained by raising the intrapulmonic pressure were also described. A preliminary attempt was made to evaluate the importance of blood pressure in the maintenance of the knee jerk. The effect of stimulation of the abdominal visceral nerves on the reflex excitability of the cord is under investigation at the present time.

On the existence of a parathyroid hormone. FREDERIC T. JUNG.

The question whether the parathyroids act by detoxication or by producing a hormone is decided in favor of the latter hypothesis by the work which various investigators have done on parathyroid extracts, provided the objection can be met that such extracts might contain artifacts which are produced by the extraction processes and which perhaps accidentally simulate some phases of parathyroid function. This objection would be met by an experiment in which the whole gland, subjected to the minimum of treatment necessary to insure its death, is administered parenterally to parathyroidectomized animals.

The present experiment was of that kind. White rats were parathyroidectomized and divided into three groups. The first group was untreated. Group 2 received, before parathyroidectomy, an implantation of dog or cat parathyroids, usually in the abdomen. Group 3 received, before parathyroidectomy, implantations of spleen, salivary gland, or testis from cats or dogs. The rats were then studied in regard to the various manifestations of parathyroid deficiency. The results from group 1 were radically different from those given by groups 2 and 3, emphasizing the fact that group 3 were the true controls. Between groups 2 and 3 a marked difference was found, particularly in the shape of the mortality curve. Numerical data were obtained which showed that the tetany was less severe, and the ultimate mortality less, in group 2 than in group 3, and the mortality curve in group 2 exhibited a striking convexity during the first 75 hours. Since the conditions of the experiment were such as to make even temporary functioning of the implants highly improbable, the conclusion is that the parathyroid tissue, while being absorbed, liberated into the body of the recipient a material which was not species-specific, not present in the control tissues, and not a chemical artifact, which did mitigate the consequences of the parathyroidectomy, and which was probably the parathyroid hormone.

Observations on the rôle of the tissues in maintaining the acid-base balance of the blood. L. N. KATZ and M. G. BANUS.

The effect of isolated muscle and the tissues of the hindleg preparation of the dog on the acid-base balance of whole blood perfused through it was analyzed following the addition of dilute hydrochloric acid. A technique was developed which made these isolated tissues comparable to denervated tissues intact in the body.

When the CO_2 tension of the blood was maintained constant, no change in pH was observed following perfusion of the acidified blood through the isolated muscle; nor was there any significant alteration in the CO_2 combining power or chloride content of the blood. *The isolated muscle apparently has no effect on the acid-base balance of acidified blood perfused through it* and yet tests showed that the amount of buffer in the muscle was sufficient to produce definitely measurable changes in the acidity and alkaline reserve of the blood. Control perfusion experiments showed that the muscle did not produce any changes in the blood which might mask its effect on acidified blood. The non-availability of the buffer material in the muscle shows that under the conditions of our experiments muscle tissue is relatively impermeable to acid and basic radicals.

When the experiments were repeated on the hind-leg preparation, an increase in pH and CO_2 combining power of the blood was observed following perfusion of acidified blood. *This indicates that the hind-leg preparation increases the buffering capacity of acidified blood perfused through it.* Direct observation showed that the change in the acid-base balance of the blood was not caused by a shift in chlorides from the blood to the tissues. The results obtained with perfusion of isolated muscle would indicate that other tissues than muscle are responsible for these changes. The exact mechanism is being investigated; at present it is not known.

The relation of the T wave to the asynchronism in the ending of right and left ventricular ejection. L. N. KATZ and S. F. WEINMAN.

The time relation of the T wave to the end of ejection in the right and left ventricle was determined under a variety of experimental conditions. The measurements were made on records of aortic and pulmonary arterial pressure curves registered by optical manometers simultaneously with the standard leads II and III of the electrocardiogram.

No consistent relation was found between the character of the T wave of the electrocardiogram and the asynchronism of the termination of ejection in the two ventricles under control conditions; nor was there any parallelism in the changes of the T wave and the asynchronism when the experimental conditions were altered.

A qualitative parallelism was found between the changes in the asynchronism of the ends of right and left ventricular ejection and the T wave when ventricular extrasystoles were induced.

When correlated with the recent tendencies in the interpretation of electrical variations, the results indicate that the T wave is not due to persistence of activity in one ventricle or the other, nor is it due to persistence at the apex or base, nor is it dependent entirely on the pathway of invasion, but rather on the non-uniform duration of activity in the various fractions of the ventricles.

The electrocardiogram is in reality the first differential quotient (comparable to a tachygram) expressing the changes in the algebraic sum of

electrical stresses in the heart from moment to moment, oriented in the direction of the lead. On this basis the T wave is the evidence of unstable electrical stress at the end of activity produced by the non-synchronous cessation of electrical activity in all fractions of the heart.

Physiological reactions induced by alpha lobelin, intravenous injections during anesthesia (preliminary communication). M. J. KING, HELEN R. HOSMER and M. DRESBACH.

Alpha lobelin is stated to be an alkaloid which acts as a specific respiratory stimulant. We found that intravenous injection of 0.08 to 0.12 mgm. per kilo caused a short-lasting (1 to 2 minutes) but pronounced increase of respiratory activity in lightly narcotized animals. In deep anesthesia, however, using the same drugs (amytal, veronal, morphine and ether), the response was poor or absent. The same was true if the experimental animal was subjected to increased intracranial pressure, carbon monoxide or carbon dioxide.

In light or moderate anesthesia a rise in arterial pressure, often marked and maintained, was noted. When, however, anesthesia was carried to the point of depressing respiration and blood pressure, lobelin caused a prolonged and dangerous fall in blood pressure in cats and dogs, with a slow recovery. Similar results were obtained in one monkey. In a normal, unanesthetized man, 0.21 mgm. per kilo intravenously resulted in dyspnea, a slow, weak pulse and pain in the cardiac region.

The similarity in the effects of lobelin and adrenin on blood pressure and respiration suggested an examination of the blood sugar. In all the animals investigated, including man, lobelin produced hyperglycemia. This problem is being further investigated.

Cerebrospinal fluid pressure and blood and cerebrospinal fluid concentration after intravenous injection of hypertonic solutions. J. M. KINSMAN, R. G. SPURLING and F. JELSMA.

In an attempt to correlate the changes which take place in the cerebrospinal fluid pressure and in the blood and cerebrospinal fluid concentration after intravenous injection of hypertonic glucose and sodium chloride solutions, a series of studies was made upon animals and man. The method of Barbour and Hamilton was used in determining the specific gravity of the whole blood, serum and cerebrospinal fluid. The following results were obtained:

1. Intravenous injection of hypertonic glucose and salt solution caused a fall in cerebrospinal fluid pressure similar in every respect to that described by Weed and McKibben and others.

2. *Blood concentration:* The specific gravity of whole blood and of serum was studied at short intervals both during and following the injection of these hypertonic solution. In some of the experiments, hematocrit and total solid determinations were made for purposes of control. There is a well marked decrease in specific gravity of the blood coming on immediately following introduction of these solutions into the blood stream. In fact, the specific gravity starts to fall rapidly while the injection is being made. This fall in specific gravity is of comparatively short duration, and a gradual upward trend toward the normal is observed during the first 20 to 30 minutes following injection. The normal was never reached, however, during the period of observation (90 minutes). Ob-

servations on the whole blood and upon the serum were in every way identical.

3. *Spinal fluid concentration:* The specific gravity of the spinal fluid shows a consistent increase following the intravenous injection of hypertonic solutions. This would indicate an actual withdrawing of water from the cerebrospinal fluid spaces into the blood stream.

The relation between capillary blood pressure and the rate of passage of liquid through the walls of single capillaries. EUGENE M. LANDIS.

The rate at which fluid passes through the capillary wall at different pressures was determined in the mesenteric capillaries of the frog by the micro-injection method previously described (this Journal, 1926, lxxv, 548). Blood-flow through a single capillary was stopped by gentle pressure with a minute glass rod. The movement of the corpuscles at the open end of the occluded capillary indicated the direction and rate at which fluid was passing through the wall. The filtering area was computed from the length and diameter of the capillary, measured by ocular micrometer. Finally a micro-pipette was introduced into the vessel to determine the capillary pressure.

At capillary pressures above 14.5 cm. of water fluid movement was always outward; at pressures below 10 cm. almost always inward, while between 10 and 13 cm. pressure there was little or no movement of fluid in either direction. The rate of filtration, measured in cubic micra of fluid per square micron of capillary wall per second, was found to be directly proportional to the difference between the capillary pressure and the osmotic pressure of the plasma colloids. The results provide direct evidence of the dependence of fluid movement on the balance between capillary pressure and the osmotic pressure of the plasma colloids.

The rôle of toxins in parathyroid tetany. E. LARSON and LEO A. ELKOURIE.

It has been reported by Dragstedt and Sudan (This Journal, 1926, lxxvii, 314) that parathyroid tetany in dogs can be controlled by oral administration of kaolin. We have fed 100 grams of kaolin, daily, to a series of twelve thyro-parathyroidectomized dogs. These animals were kept on a diet of kitchen scraps which consisted very largely of carbohydrates. We found it necessary in every case to resort to frequent injections of calcium salts in order to prolong the lives of the animals. In order to better compare the rapidity and severity of the tetanic attacks when relieved by intravenous injections of calcium lactate, eight dogs were operated and the tetany controlled by this method. Analyses of the blood at the time of tetany has always shown a low value for calcium and usually a decrease in guanidine content.

Kaolin does not absorb any guanidine from a 0.2 per cent hydrochloric acid solution. From an alkaline solution there is no absorption for at least a period of one hour. The calcium content of kaolin is negligible.

From our results we conclude that kaolin does not have any decided effect in controlling tetany in thyro-parathyroidectomized dogs.

Basal metabolism in the rat during the oestrous cycle. MILTON O. LEE.

The basal metabolic rate was determined at different stages during the oestrous cycle in eight female albino rats from four to seven months of age. At least one complete cycle was followed in each rat and from one to twenty

determinations were made in each stage. A modification of the gravimetric method of Haldane was used. Respiratory quotients were determined during two complete cycles in two rats and found to be nearly constant for fasting animals; thereafter an R.Q. of 0.75 was assumed. Body surface was computed from the formula $S = kW^{\frac{2}{3}}$, in which S is the body surface in square centimeters, W is body weight in grams, and k is 11.36. The heat production per square meter of body surface per day was found to show consistent variations from the general mean only toward the end of stage V (dioestrum) and at the beginning of stage I (pro-oestrum). At that time an average increase amounting to approximately 12 per cent of the general mean was noted. This increased heat production appeared in the last ten hours of the dioestrum and gradually disappeared in the early part of the pro-oestrum. During none of the other stages of the cycle was there any significant change in the heat production. Even during stage II (oestrus) which is characterized by a marked increase in excitibility and voluntary activity, the average heat production was below the general mean. These results are interpreted as indicating that the oestrus-producing hormone of the ovary is not primarily a metabolic stimulant. The increased heat production in the rat toward the end of the dioestrum seems to be comparable to a slight increase reported in women at menstruation, and is ascribed to an effect of an ovarian hormone on the thyroid. The metabolic rate was determined in these same rats following ovariectomy, at intervals over a period of two months. The mean of these determinations was about 10 per cent below the mean of the determinations before removal of the ovaries.

Diurnal variations in the blood specific gravity and erythrocyte count in healthy human adults. CHAUNCEY D. LEAKE, MARTHA KOHL and GEORGE STEBBINS.

With the falling drop method of Barbour and Hamilton for the determination of blood specific gravity, and with observations at hourly intervals, the average maximum diurnal variation in six healthy men was 0.0033, and in seven normal women 0.0027. The general average of 118 observations in these men was 1.0565, and in 92 observations in the women it was 1.0533.

In these same individuals, the average maximum diurnal variation in the red blood cell count was 345,000 for the men, and 310,000 for the women. There was no direct correlation between the changes in specific gravity and erythrocyte count of the blood, except a slight simultaneous fall after meals, probably due to blood dilution.

These persons were students in good health, from 18 to 30 years of age, and were not involved in any strenuous or abnormal tasks on the day of observation.

The leucocytic resistance to hypotonic saline solutions in healthy men. C. D. LEAKE and P. K. KNOEFEL.

The determination of the relative number of fragile leucocytes by the method of Sampson (1) has been found satisfactory, except that it is not necessary, in the dilutions used, to include sodium citrate in the diluting fluid to prevent coagulation. The leucocytic resistance to hypotonic saline solutions by this method was determined in 25 healthy men from 18 to 30 years of age. There was considerable individual variation, the resistant

leucocytes ranging from 18 to 43 per cent, and the fragile leucocytes from 57 to 82 per cent. The average for the resistant white cells was 32 per cent, and for the fragile ones, 68 per cent.

(1) SAMPSON, J. J. 1924. Arch. Int. Med., xxxiv, 490.

The development of "experimental neurasthenia" in the sheep during the formation of difficult conditioned reflexes. H. S. LIDDELL and T. L. BAYNE.

Pavlov (1) has pictured the normal activity of the cerebral hemispheres as a delicate equilibrium between localized processes of excitation and inhibition. He has found in the dog that if tasks of too great difficulty are imposed a derangement of this equilibrium will occur with the predominance of either excitation or inhibition. The abnormal state of the nervous system in which exitation prevails has been called experimental neurasthenia. We have observed the onset of this condition in a sheep in which, during a period of almost four years, both maze habits and simple conditioned reflexes had been established. With the metronome beating once a second this animal received an electric shock on the foreleg at the sixth beat. A leg movement at the sixth metronome beat without the shock—a delayed conditioned reflex involving inhibition which is very easily established in the dog, proved a difficult task for the sheep. After ninety-eight combinations of metronome and shock (fifteen per day) a delayed response was quite regularly obtained. We then attempted to hasten the stabilizing of the reflex by increasing the number of combinations to twenty per day. On the second day, however, the animal's behavior showed a striking change. Previously it was docile and remained quietly in the harness but now it resisted being led to the experimental room and during the experiment it was in almost constant movement. Furthermore, the delayed reflex was replaced by an exaggerated leg movement to each metronome beat. The inhibition upon which the delayed reflex depends had evidently disappeared. The nervousness increased even when the number of tests was reduced to five per day. After three days' rest the delayed reflex returned only to disappear again the next day and the nervousness increased. The conditions necessary for recovery from this abnormal state are now being investigated. It is interesting to note that a thyroidectomized sheep under like conditions of training failed to exhibit this nervous disturbance.

(1) PAVLOV, J. P. Die höchste Nerventätigkeit (das Verhalten) von Tieren. Bergmann, 1926, p. 313.

The respiration and lactic acid metabolism of excised testicular tissue. ROBERT O. LOEBEL,[1] and R. A. HICKLING.

In frog's muscle there is excellent evidence of a complete hexose $\rightleftarrows$ lactic acid cycle which is intimately related with the respiration so that four molecules of lactic acid are synthesized when one molecule of lactic acid, or its equivalent, is oxidized. The significance of these processes has recently been shown for malignant cells. With important mammalian tissues like liver, kidney, and even muscle, the data are much less conclusive.

[1] Part of this work was done under a Fellowship of the National Research Council and part under a grant from the American Medical Association.

We extended these studies to excised testicular tissue derived from the rat; first, because this tissue in its metabolism, and in preserving the capacity to proliferate in the adult animal seems to be intermediate between normal and malignant cells; and secondly, because, unlike liver or Jensen sarcoma cells, for instance, it must, very soon after excision, draw its foodstuff from the medium in which it is immersed, and therefore, the effect of various foodstuffs can be readily investigated.

After killing a rat by decapitation it is possible to obtain with practically no local trauma strands of tubules which are very fine and can be readily provided with an adequate oxygen supply in a phosphate-buffered Ringer solution. After a series of experiments which demonstrated that sodium lactate of itself did not diminish the anaerobic glycolysis, simultaneous measurements were made of oxygen consumption, carbon dioxide production and lactic acid formation, with and without the addition of M/40 sodium lactate, on tissue obtained from the same animal. The determinations were carried out by the manometric methods of Warburg and Meyerhof.

With no foodstuff in the medium, respiration is low and falls rapidly. With lactate in the medium the oxygen consumption is greater and remains at almost the same level for several hours. There is a disappearance of lactate from the solution. The respiratory quotient shows a definite rise. When these figures are compared with each other it is obvious that the high efficiency of the lactic acid transformation in frog's muscle cannot be achieved by testicular tissue in Ringer's solution; at most there is a synthesis of carbohydrate in moderate excess of what is removed by oxidation. These studies are being continued using serum as medium.

Some effects of hemorrhage, low oxygen, cyanide and sodium bicarbonate and carbonate on expired oxygen and carbon dioxide as recorded with continuous electrometric methods.[1] DANIEL A. MCGINTY and ROBERT GESELL.

Variations in expired oxygen and carbon dioxide were followed during uniform artificial ventilation with pneumothorax.

Hemorrhage increased the expired oxygen and decreased the expired carbon dioxide. Subsequent injection reversed these effects. A supernormal phase of excess absorption of oxygen and excess elimination of carbon dioxide obtained for several minutes following reinjection. With prolonged hemorrhage the initial decrease in oxygen absorption frequently gave way to a slowly progressive increased absorption. This was finally augmented by reinjection. A corresponding increase in carbon dioxide elimination occurred. The magnitude of the effects of hemorrhage showed large variations. In general animals were sensitive to minor changes in blood volume. In some experiments 20 cc. of hemorrhage reduced gaseous exchange while in others 200 cc. or more (14 kgm. average weight of dogs) produced only small effects. Injection of saline solution improved gaseous exchange almost as much as blood.

Administration of air poor in oxygen produced a simultaneous decrease in oxygen absorption and carbon dioxide elimination. The magnitude of the change varied with the reduction of oxygen in the administered gas. Readministration of room air reversed the effects. A supernormal phase of excess gaseous exchange lasting several minutes followed the readminis-

[1] Gesell, Robert and Daniel A. McGinty. 1926. This Journal, lxxix, 72.

tration of room air. This may be missing if oxidations are seriously disturbed and if recovery is poor. Prolonged administration of air poor in oxygen is frequently accompanied by a slow improvement of gaseous exchange after the initial reduction.

Intravenous injection of sodium cyanide elicited a simultaneous decrease in oxygen absorption and decrease in carbon dioxide elimination. With good recovery a supernormal phase occurred as in the hemorrhage and low oxygen experiments. With large injections the gaseous exchange may be permanently impaired.

Intravenous injection of sodium carbonate elicited a temporary increased absorption and utilization of oxygen accompanied by retention of carbon dioxide. Injection of sodium bicarbonate elicited a temporary increased absorption and utilization of oxygen accompanied by an increased carbon dioxide elimination. In some experiments increased oxygen consumption and increased carbon dioxide elimination were approximately proportionate. In other experiments carbon dioxide elimination increased out of proportion to the increased oxygen consumption. The results agree with the acid stimulating effect of sodium bicarbonate on the respiratory center; that is, the intravenous injection of sodium bicarbonate hampers the movement of acid from the tissues into the blood and accelerates the movement from the blood into the alveolar air.

The data on expired gases so far collected support the view of the importance of cellular acidity on respiratory control. They show the significance of altered blood flow, disturbed coördination of the dual function of hemoglobin, and impaired oxidation on cellular acidity. They agree in full with changes in blood acidity studied under like conditions and substantiate the explanation of these changes.

Rheotropism in Fundulus—A forced movement. ROLLAND MAIN.

Fundulus responds to a moving environment by nystagmus and a tonus change in the tail muscles, which causes its orientation in the direction of the environment. This forced movement is often seen in fishes although the swimming movements necessary to orient the fish are absent. The fish tend to respond to the images seen in the front part of the visual field.

This tonus change is probably due to the proprioreceptors of the eye muscles, for there is a distinct relationship between the movement of the eyes and the degree of tonus in the tail, whether the eye movement be forced or passive.

This forced movement replaces Lyons' suggestion of the rheotropic stimulus by a moving retinal image.

The specific dynamic action of glycocoll and alanine with special reference to the dehepatized animal. FRANK C. MANN, CHARLES M. WILHELMJ and JESSE L. BOLLMAN.

A study is in progression on the effect of the intravenous injection of amino acids on the gaseous exchanges and nitrogen elimination in dogs. Observations have been made on normal animals in order to obtain control data for a similar study on the dehepatized animal. It has been found that the intravenous injection of glycocoll and alanine on the normal animal produces a definite specific dynamic action accompanied by the usual change in the nitrogen elimination in the urine which follows the

injection of amino acids. Thus far it has not been possible to obtain a specific dynamic action of the amino acids in the dehepatized animal.

The excess metabolism and muscular efficiency for moderate work. M. ELIZABETH MARSH.

A study of the character of the metabolism during a very mild or moderate piece of work as determined by the respiratory quotient of the excess metabolism, measured during the period of work and complete recovery therefrom shows wide variations in the same individual at different times and between different individuals, confirming the belief expressed by Campbell, Douglas and Hobson. In certain individuals the R.Q. during the work and recovery period rises sufficiently so that the R.Q. of the excess metabolism is in the neighborhood of 1.00. On the other hand others in a long series of experiments show almost invariably a drop in the quotient during work and recovery giving therefore a low quotient of the excess metabolism, an average of seventeen experiments upon one individual being 0.78; of 33 experiments upon another is 0.78 for one year and 0.89 the following year in 11 experiments. Still others apparently change the character of their metabolism little if any during a light piece of work.

A subject on a controlled diet shows much less variation. Table 1 shows the R.Q. and the excess metabolism in uncontrolled mixed diet to be 0.95 for an average of 16 experiments. On the controlled diets 0.95 for 11.2 cal. of work on the carbohydrate and 1.00 for 8.8 cal. of work; while on a fat diet it is 0.83 for the more severe work and 0.80 for the lighter work.

TABLE 1

SUBJECT	DIET	WORK (CAL.) (AVERAGE)	R.Q. OF EXCESS METABOLISM
B. C.	Normal	8.4*	0.95
	Carbohydrate	11.2	0.95
	Carbohydrate	8.8	1.00
	Fat	11.2	0.83
	Fat	8.8	0.80

* Varied between 4.5 and 13.70 Cal.

The net efficiency of the subject upon this diet of carbohydrate and then of fat shows a progressive decrease in the fat diet as compared with the carbohydrate and mixed diet, but there is only a slight drop at the end of three days and not until the end of eleven days was there the 11 per cent difference noted by Krogh and Lindhard.

Diastolic size of the heart during and after exercise. F. D. MCCREA.

The only experimental evidence upon the changes in heart size during dynamic exercise is that of Nicolai and Zuntz and Meek and Eyster. These investigators found slight increases during, and more marked decreases after exercise, although exceptions were noted in individual cases.

Teleroentgrams were taken before, during, and after moderate and severe exercise. A bicycle with prony brake was used. Venous pressures and pulse rates were determined at approximately one minute intervals. Diastolic volume was computed from Bardeen's table.

The results show that the effect of moderate exercise for 10 minutes on the heart is variable and is characterized by little or no change in diastolic size. After exercise the heart was the same size or slightly smaller. All subjects during 10 minutes of severe exercise showed evidences of slight increases in area, though here again the volume changes were small. A slight decrease was noted in every case save one after exercise was over. One subject during 30 minutes of severe exercise shows more marked increase of diastolic size, without return to normal size 5 minutes after cessation of exercise.

The data seem to show a relationship between venous pressure, pulse rate, and cardiac volume. With high pulse rates the tendency is for venous pressure to rise less high, and for cardiac volume not to increase. With slower pulse rates there is usually associated a higher venous pressure and an increase of diastolic size.

The elevated narrow-path maze for studying the effect of various substances on rats. W. R. MILES.

If an animal is injected with any substance and then placed back in his cage among other animals only a very rough estimate can usually be made of the intensity of the effect on his behavior. More trustworthy results may be gotten by first permitting the animal to learn a fairly complicated path in going to food and later using this behavior as a test instrument. The elevated maze is a simple inexpensive means to this end. It is a path 1 inch wide, 30 inches above the floor, complicated by many right-angle turns and many dead ends (blinds which lead nowhere), and the path is without sides or walls thus assuring easy observation of the animal. The path is made up of unit frames each 3 feet long which are set side by side to construct the desired pattern. Modifications in arrangement are easily made and the units stack compactly for storage between tests. The narrow width requires of the animal a certain nicety of coördination in walking or running. Normally animals do not fall off or climb down. Time, errors and other features of the runs are recorded.

The effect of chemicals on form and locomotion in Amoeba proteus. S. O. MAST.

It is generally assumed that the form of Amoeba is specifically related to the chemical content of the surrounding medium and that locomotion is possible only in solutions which contain certain salts especially calcium. The following results seem to indicate that these assumptions are not valid.

If Amoeba proteus is transferred from culture medium to distilled water it soon becomes nearly spherical in form but after a few moments 5 to 15 new pseudopods appear. These rapidly extend in all directions until they are relatively very long and thin, then movement ceases. Two to ten hours later movement begins again. At first it is slight and sporadic, appearing now in one pseudopod and then in another. Soon the tip of some of the pseudopods enlarges and one of them become attached to the substratum, after which the central substance begins to flow into this attached pseudopod. This continues until the amoeba has become a flattened lobose form which moves freely in close contact with the substratum, then it gradually elongates and becomes more and more regular in form until it is nearly ellipsoid in shape and has but one pseudopod.

Later it again becomes more irregular in shape and more sluggish and sporadic in its movements until it finally comes to rest and dies after having been in the distilled water 6 to 9 days.

All of the phenomena described occur in the purest water that can be produced in a tandem pyrex glass still and in dishes lined with paraffin in which the distilled water is frequently changed so as to avoid accumulation of waste products from the animal. They also occur if carbon dioxide is excluded. However, if in place of leaving the amoebae in distilled water after they have become radiate, they are transferred to N/1000 NaCl, KCl, LiCl, NH_4Cl, $CaCl_2$, $MgCl_2$, $CaSO_4$, $Ca(NO_3)_2$, $Ca_3(PO_4)_2$, $Ca(C_2H_3O_2)_2$, Na_2SO_4, $NaNO_3$, $Na(PO_4)_3$ or $NaC_2H_3O_2$ the time required for attachment and the beginning of locomotion is reduced from several hours to a few minutes. Similar results were also obtained in weak solutions of acids and alkalies. But sugar solution were found to act like pure water.

It is consequently evident that, while the results obtained indicate that locomotion and all the different forms ever assumed by an amoeba occur in pure water, certain chemicals greatly affect the rate of some of the processes associated with locomotion and with change in form. And the fact that all of the various electrolytes tested act essentially the same indicates that the effect produced is in some way associated with the action of the ions on the electric charge on the surface of the amoeba.

The temperature of the air in contact with the skin of the forearm. B. McGlone and H. C. Bazett.

Constantan-manganin thermocouples of light wire (0.08 mm. diameter) were mounted vertically above the bare arm so as to measure the temperature gradients existing in the direction of the convectional currents. By means of a screw of 0.5 mm. pitch the thermocouple employed was raised and lowered in steps of this amount. The temperatures of the air were determined from the galvanometer deflections recorded photographically. The arm was bared one or more hours prior to the first thermoelectric measurement and during the period of observation it was immobilised in an arm rest. Various environmental conditions were employed. These were expressed in terms of "effective temperature (basic scale) factor," (F. C. Houghten and C. P. Yagloglou, Trans. Amer. Soc. Heat and Vent. Eng., 1923, xxix, 361). To exclude the effect of a slight temperature lag of the thermocouples and their support, the mean of the temperature determinations at identical distances was evaluated from the values obtained on raising and lowering the thermocouple.

Although a uniform type of the temperature gradient exists, the slope has varied with the dry bulb temperature, and the amount of air movement. The results show that in environmental conditions of high dry bulb temperature and little air movement the temperature of the air at a distance of 5 to 6 mm. from the surface of the skin either unshaved or shaved is 1.0°C. or less below that of the surface temperature. With a lower dry bulb temperature and an increase in air movement, the gradients are steepened, but even under extreme conditions the temperature at 0.5 mm. distant has not been more than 1.0°C. below that of the surface. The protection afforded even by the small hairs present is quite marked; when gradients over unshaved and shaved areas are compared definite differences can be distinguished.

A study of hemophilic blood by plant physiological methods. D. I. MACHT.

The author has called attention elsewhere to the usefulness of plant test-objects in studying certain blood conditions. Thus while the blood from patients with pernicious anemia produces no definite effect on animal test-objects it was demonstrated that such blood is certainly toxic to the growth of certain seedlings. This effectiveness on the part of seedlings has been utilized by the author in part for a differential diagnosis of pernicious anemia from other blood conditions. Inasmuch as the plant physiological method of testing has given the first experimental proof of the poison of some toxic constituent in the blood for pernicious anemia it was deemed worth while to test the effect of blood serum in other blood diseases in the same way. In the present work specimens of blood were obtained from three different patients suffering from uncomplicated hemophilia. The blood serum from these specimens was tested repeatedly by the author's method on the growth of the seedlings of Lupinus albus. It was found that they were in no way toxic to the plant protoplasm but behaved exactly like normal blood serum. These findings speak in favor of Howell's theory concerning the etiology of hemophilia.

Studies on vigor. IX. The effects of ovarian extirpation on fatigability of muscle in the rat. HUGH H. MILEY.

Studies on female albino rats by the revolving cage method have shown that spaying results in a marked decrease in voluntary activity. Available data offer little in the way of explanation of such findings. Believing that the field could be narrowed somewhat by a quantitive study of the fatigability of nerve-muscle preparations, in situ, a comparative study was made of the strength and fatigability of the gastrocnemius muscles of ovariectomized and normal rats. The animals were anesthetized with amytal, which was injected subcutaneously so that uniform and prolonged anesthesia could be obtained. The muscles were stimulated through their motor nerves at one second intervals, using break shocks only. It was found that ovariectomized animals gained weight more readily than their controls and the weights of the individual muscles were greater in the spayed animals. The absolute strength per gram of muscle averaged 40 per cent greater for the controls than for the experimentals. The proportion of gastrocnemius weight to total body weight averaged the same in each group. The total work performed by the ovariectomized animals was only 33.6 per cent of that done by the controls. This agrees well with the decrease in voluntary activity following spaying. The average daily activity of the castrated males in Gans and Hoskins' experiments was 2298 revolutions while that for the spayed females in these experiments was 2651 revolutions. A comparison of the ergographic and revolving cage records shows that the best control did 2,663,900 gram-centimeters of work correlated with a daily activity of 10,091 revolutions. The best experimental did 893,600 gram-centimeters of work and had a daily activity record of 683 revolutions.

The functions of the cerebellar nuclei as determined by faradic stimulation. FREDERICK R. MILLER and N. B. LAUGHTON.

In these studies, which were made on the cat, decerebration, performed in deep anesthesia, was through a plane lying dorsally just in front of the superior colliculi and ventrally in front of the infundibulum; the nuclei

rubri were thus preserved intact, an essential condition in stimulating the cerebellar nuclei. The dorsal surfaces of the nuclei were exposed by removing, with a horizontal slice, part of the cerebellum to a suitable depth. Unipolar faradisation was then applied to the various nuclei, the approximate positions of which were determined by measurements. The place stimulated was marked with a small bristle and Weigert sections were subsequently made to determine whether the particular nucleus had in reality been stimulated.

The following responses are characteristic of the nuclei:

Nucleus emboliformis and *n. globosus*, both yielding approximately the same reaction: marked flexion of ipsilateral foreleg; inhibition of decerebrate tonus of contralateral foreleg; flexion of ipsilateral hindleg; curvature of body; ocular movements.

Nucleus dentatus: flexion, sometimes repeated, of ipsilateral foreleg; palmar flexion in contralateral foreleg; hindlegs more rigid; curvature of body.

Nucleus fastigii: strong flexion of both forelegs; flexion of ipsilateral hindleg.

Simultaneous records of the biceps and caput laterale of the triceps in the ipsilateral foreleg were obtained on stimulating the nuclei; the remaining muscles of the limb were denervated. The records revealed the following features:

Nucleus emboliformis: during stimulation, contraction of biceps and relaxation of triceps; after stimulation, relaxation of biceps and "rebound" contraction of triceps.

Nucleus fastigii: effects as regards these two muscles were similar to those from n. emboliformis. The reactions of these muscles thus follow the principle of reciprocal innervation.

The efferent pathways for the reactions of the lateral nuclei are through the brachium conjunctivum, nucleus ruber and rubrospinal tract, possibly also through the rubroreticular tract. The principal efferent pathway for reactions of the nucleus fastigii is through the fastigiobulbar tract (fasciculus uncinatus of Russell).

The reactions of the cerebellar nuclei are changes in postural tone with augmentor and inhibitory components; they are of a coördinated character, involving reciprocal innervation of antagonistic muscle groups. The nuclear reactions, taken with the inhibitory capacity on decerebrate tonus possessed by the rostral cerebellar cortex, constitute the evidence for the view already proposed by one of us (Physiol. Rev., 1926, vi, 124) that the cerebellar influence on tone is of both a positive and negative kind.

The influence of rate and irregular action of the heart on coronary flow. G. H. Miller, Fred M. Smith and V. C. Graber.

The influence of cardiac rate on coronary flow was studied in both the isolated and the intact heart. In the former, the heart of the rabbit was driven at different rates by means of rhythmically induced break shocks. In every instance an increase in the heart rate was associated with an augmentation of the coronary flow. When the initial cardiac rate was very fast, further acceleration did not produce significant changes in the rate of coronary flow. The greatest increase in coronary flow was obtained with a moderate acceleration of cardiac rate, in hearts whose initial rates were relatively slow.

Further studies were carried out on the intact heart of the dog. The coronary flow was determined by introducing a Morawitz-Zahn cannula into the coronary sinus. The blood volume was maintained by reintroducing the blood at a uniform rate through a cannula in a femoral vein. Heparin was used to prevent coagulation.

Changes in cardiac rate were produced by warming and cooling the sinus node and by stimulation of the vagus nerve. The decrease and increase in heart rate were associated respectively with a reduction and an augmentation of coronary flow. In the experiments in which cardiac rate was decreased by stimulating the vagus, the reduction in coronary flow was striking.

Irregular action with premature contractions produced by stimulation with single induction shocks did not cause any significant change in rate of coronary flow. Auricular fibrillation gave varied results. In some instances there was practically no change in coronary flow, while in others there occurred a slight augmentation or a reduction in rate of flow. In those experiments in which a rapid and more regular cardiac action followed the faradic stimulation of the auricles the rate of coronary flow was usually increased.

The effect of calcium on the toxicity of carbon tetrachloride in dogs. ANNE S. MINOT.

The oral administration of even very large doses of carbon tetrachloride to dogs on a well balanced diet produces no outward signs of intoxication. Liver function tests, especially the determination of bilirubin in the blood serum, show that some temporary damage has been done to the liver.

When dogs are on a low calcium diet similar doses of the drug produce about the same disturbance in liver function, but death in coma or tetanic convulsions occurs in most cases 48 to 60 hours after the carbon tetrachloride is administered.

These symptoms may be relieved by repeated intravenous injections of calcium chloride or by oral administration of ammonium chloride.

Death seems to be due to a lack of calcium ions in the blood secondary to the bilirubinemia caused by the liver damage produced by the carbon tetrachloride.

The influence of liver and meat diet on blood regeneration. GULLI LINDH MULLER.

Pigeons, when subjected to simple starvation, fresh water being given every day, show an increasingly severe anemia with comparative aplasia of the radial bone marrow. Feeding will cause blood regeneration in a short time.

A series of pigeons was starved for varying periods of time. The decrease of erythrocytes and hemoglobin, as a rule, was directly proportional to the loss of weight. The effect of broiled beef liver, broiled beef meat, and pigeon feed on the blood regeneration was studied.

In interpreting the data two factors must be considered: namely, the replacement of lost weight and the regeneration of the blood. Pigeons fed grain, their usual food, are taken as a standard. The weight, red blood cells, and hemoglobin of grain fed animals were regained in a short time. The liver fed pigeons regained their initial weight in thirteen to twenty days. The highest number of red blood cells and hemoglobin

was reached in about twelve days, ranging from 3,000,000 to 3,600,000 erythrocytes, and 76 to 79 per cent hemoglobin (average normal values 4,000,000 erythrocytes, 95 per cent hemoglobin). Then the red blood cells began to decline steadily and when the erythrocytes were approaching 2,000,000 cells and the hemoglobin 50 per cent, the previously good appetite began to fail. Animals were killed before any appreciable decline of weight had taken place.

In contrast to this, meat fed pigeons regained their weight, erythrocytes, and hemoglobin as rapidly as, and in some instances more rapidly than, control pigeons.

The bone marrow of liver fed pigeons showed a marked depression of the megaloblastic or earliest stage of the erythrocytic series in contrast to the grain and meat fed pigeons, in which megaloblasts were abundant. The cholesterol was increased to five times the normal in the liver fed animals.

It is suggested that the depression of blood formation in pigeons may be due to an inhibitory substance in the liver. This inhibitory effect is perhaps the beneficial factor in the liver diet treatment of pernicious anemia in which hyperactivity is present in the earliest forms. As to the nature of this inhibitory substance the increase of the cholesterol in the serum is suggestive. Whether the substance inhibiting the red blood cell formation in pigeons is identical with the substance effective in pernicious anemia, or with cholesterol, only the future will show.

The effect of injury to the spinal cord of rats in prenatal stages. DAVENPORT HOOKER and J. S. NICHOLAS.

The experiments of the senior author have shown that injury to the cord in young rats may be followed by later adjustments of the nervous reactions so that the loss of function caused by the injury is not nearly so great as was initially expected.

Gerard and Koppanyi have reported regeneration of cords in rats which were operated upon during fetal life. Their work is subject to criticism since there was no histological control and also since positive identification of the operated animals was not secured.

Our experiments have been performed upon rat fetuses from twelve to nineteen days of age, the animals being either removed at various intervals or permitted to remain in utero until birth. The animals were positively identified by cutting off the forelimb according to the method developed by the junior author. Histological studies were made upon each case after the extent and range of movements had been tested.

Fourteen cases in which either partial or complete destruction by cautery of a portion of the cord had been secured fail to show the slightest signs of regenerative reconstitution of the nervous system. Slight tendencies toward spasticity and slight variations from definite types of response were observed but the reactions are surprisingly normal.

Observations upon temporal summation and upon inhibition of the crossed extensor reflex before and after deafferentation. J. PI-SUÑER and J. F. FULTON.

In decerebrate cats after successful deafferentation of the vastocrureus muscle, the response (electrical and mechanical) to the second of two equally intense break-shock stimuli applied to the contralateral sciatic nerve during the relaxation of the response evoked by the first stimulus is,

in the majority of preparations, more ample after deafferentation than before. This is interpreted as due to the absence, after posterior root section, of the proprioceptor inhibitory influence which in a normal muscle originates from sudden onset of active contraction. The deafferented quadriceps is usually more readily inhibited by flexor traction and by single break-shocks (applied to the ipsilateral sciatic nerve) after deafferentation than before.

Further studies on the reflex mechanism of the empty stomach of monkeys. T. L. PATTERSON.

Previous observations on Macacus and Ringtails monkeys reported before this Society have shown that the contractions of the empty stomach are practically identical with those of man. Furthermore, central stimulation of the vagus and sciatic produces certain reflex effects on this organ as has been exhibited in acute experiments with the animals under light ether anesthesia. The balloon method was used and a cannula was introduced into a femoral artery for simultaneously recording the blood pressure. An extension of these latter experiments has shown that stimulation of the central end of a vagus or a sciatic may lead to either an augmentation or an inhibition of the stomach, and that the reaction appears to be dependent primarily on the existing state or condition of the gastric musculature itself, that is, whether it is in a state of activity (hypertonus) or in a state of rest (hypotonus). If the stomach is hypertonic reflex inhibition is more apt to follow central stimulation of these nerves, while if it is hypotonic a contraction may occur or there may be a definite increase in the tonus. Therefore, the effect may involve both tonus and movement.

Changes in permeability associated with the absolute minimum vital temperature. NELLIE M. PAYNE.

Associated with the absolute minimum vital temperature or the lowest temperature at which organisms can live are, in the case of insects, certain definite changes in cell permeability. The cell walls become permeable to protein. Somewhat later rapid oxidation takes place especially in the prothoracic region. Death, either local or general, follows oxidation. Water can leave the cells during the freezing process and reënter. Apparently changes in permeability to protein are irreversible.

The tonic contraction of decerebrate rigidity compared with nerve-muscle response in the same muscle. E. L. PORTER.

The small muscles elevating the last few vertebrae of the tail in the decerebrate cat were employed. When the nerve to one of these muscles is stimulated by single shocks of gradually increasing strength, the response of the muscle is by contractions occurring in groups, the height of which increases in a step-like manner, with no gradation between the successive steps.

In agreement with previous observers, it is believed that in such a record each step in contraction height represents the activity of a single motor neurone and the muscle-fibers innervated by it. This is the neuromuscular unit of contraction.

If now the same muscle instead of being stimulated artificially is caused to contract tonically, i.e., to pass into decerebrate rigidity, the increase in contraction height is again by a series of definite steps or increments which

are of the same order of magnitude as those produced by the stimulation of the motor nerve.

This result favors the view that the same neuromuscular units are employed in the contraction of decerebrate rigidity as are thrown into action by artificial stimulation of the motor nerve.

Observations on the effects of protein split-products upon metabolism, and their relation to the specific dynamic action of protein. DAVID RAPPORT and H. H. BEARD.

Previous work has shown that proteins, irrespective of their amino acid content, have approximately the same specific dynamic action. This is in apparent conflict with the theory that glycine and alanine are the chief causes of the stimulating effect of the proteins on metabolism. The present communication attempts to deal further with this discrepancy. It is found that "fraction I" of the hydrolysis of gelatin (namely, the fraction extracted by and precipitated in butyl alcohol, and consisting of the mono-amino, mono-carboxylic acids) has a slightly greater influence on the metabolism of a dog than has an equivalent amount of gelatin itself; and that the corresponding fraction of casein has a considerably larger effect than has its parent protein. The effect on metabolism of "fraction I" is proportional to the amount ingested. The specific dynamic action of glycine is not neutralized by simultaneous ingestion with "fraction I." In this respect, the fraction differs from protein. The effects of a protein and "fraction I," when these are administered together, are summated.

"Fraction I" of the casein hydrolysis has a greater influence on metabolism than has the corresponding fraction of gelatin. Since the former contains practically no glycine and very little alanine, a search for the possible causes of the activity of this fraction of casein has been made. Theoretical considerations indicate that of the other amino acids, only valine, phenylalanine and tyrosine could be of importance in this connection. Valine was found to have no effect on the heat production. Phenylalanine, however, has a powerful specific dynamic action—greater than that of glycine, per gram ingested. Tyrosine had an effect, in the animal studied, comparable to that of alanine. Per gram metabolized, using the inexact criterion of the urinary N excretion to determine the amount of amino acid destroyed, it is even more powerful than phenylalanine.

On the basis of results obtained with the individual acids, it is calculated that the five amino acids known to have a specific dynamic action—namely, glycine, alanine, leucine, phenylalanine and tyrosine—can account for the effect of "fraction I" on the heat production in the case of both gelatin and casein, but that in the latter, phenylalanine and leucine account for most of the stimulating effect, rather than glycine and alanine.

Transferring these calculations to the proteins themselves, it is found that while the five amino acids enumerated above can apparently account for the effect on metabolism of certain proteins, such as beef protein and gelatin, they do not suffice to explain the specific dynamic action of other proteins, such as casein and gliadin.

Factors influencing the anaerobic activity of cardiac muscle. ALFRED C. REDFIELD and JOHN T. EDSALL.

Cardiac muscle of the turtle, isolated and contracting isometrically in the absence of oxygen fatigues according to the law that each systole

diminishes the magnitude of the stress which is developed as the result of the next systole by a constant fraction of itself. If S_a is the stress developed at any time in the course of the development of fatigue and S_b is the stress developed at any subsequent beat and b is the number of intervening beats, then

$$\log_e S_a - \log_e S_b = \lambda_1 b$$

The constant λ_1 has a value fluctuating between 0.003 and 0.010 in ventricular muscle stimulated 10.7 times per minute. It expresses (1) the degree to which each beat of the heart diminishes the force of the subsequent beat and (2) the fraction of the total subsequent anaerobic activity which is realized by any single systole. Because its value is independent of the degree of fatigue its determination provides a valuable method of measuring the effect of any factor on the anaerobic processes in muscular contraction.

Changes in the hemoglobin content of the blood of dogs following splenectomy. G. B. RAY and B. STIMSON.

Following the removal of the spleen there is a gradual decrease in the pigment of the blood as determined by Stadie's cyanhemoglobin method. The decrease in oxygen capacity of the blood is more rapid than that of the total blood pigment with the result that one finds an accumulation of nonfunctional blood pigment which reaches a maximum in about 3 to 4 weeks. Apparently the first step in the destruction of cells following splenectomy is the loss of oxygen-carrying power.

On the interpretation of the electromyogram from voluntary and reflex contractions. CURT P. RICHTER.

Previous workers have been unable to establish any essential differences in the electromyograms from reflex and voluntary contractions. Their investigations, however, have been confined chiefly to the reflex postural contractions of decerebrate animals. We have attacked the problem from a different angle, and have recorded action currents set up by the grasping reflex of the new-born human infant. Such a method of approach is especially favorable for purposes of comparison, since this reflex activity involves the same muscles as are used in voluntary grasping in the adult. We employed in these experiments special electrodes, and a special technique in attaching them to the body. The action currents so obtained are free from "Nebenzacken" and secondary waves, and occur at frequencies between 60 and 75 per second. They are quite as regular in both amplitude and frequency as those currents recorded by Cooper and Adrian from reflex contractions caused by rhythmical induction shocks applied to the central end of a cut afferent nerve. The fact that these large waves are present long before voluntary activity begins suggests very strongly that they are produced by impulses set up in the reflex arc by proprioceptive end-organs and not by impulses coming down from higher centers.

The influence of activity on the Manoilov reaction in blood and tissue extracts. OSCAR RIDDLE and WARREN H. REINHART.

The blood of both male and female pigeons gives a lighter color (male reaction) in active than in inactive stages of the reproductive cycle. Blood from birds made to fly 10 minutes usually gives a lighter color than samples taken immediately before this period of exercise, but this result

is probably influenced by blood lost for first sample. Blood from younger birds gives a lighter color than is obtained from older ones. Aqueous extracts of active tissues (muscle, ovary, heart, gizzard) usually yield a lighter color than tissues presumably less active (liver, egg-yolk). The three glands of the oviduct each gives lightest color when actively secreting, and progressively more color at stages more removed from active functioning. Extracts of whole embryos give lightest color when prepared from freshly killed embryos; decidedly darker color when obtained from embryos dead 1 to 3 days. These results reveal new precautions necessary for comparisons made with this test, and provide further evidence that the reaction is primarily a better indicator of metabolic rate than of sex.

A roentgenologic study of gastric hunger motility in man. F. T. ROGERS and C. L. MARTIN.

A method has been devised whereby the gastric hunger contractions of man which have hitherto been studied by the rubber balloon and graphic registration methods, may be simultaneously observed fluoroscopically or may be radiographed. Observations have been made on four healthy young men after periods of twenty-four hour fasting. It is now possible to give a direct description of the contractions classified by Carlson as "twenty seconds rhythm," and contractions of types I, II and III.

The twenty seconds' rhythm is, as Carlson suspected, simple peristalsis. In type I contractions there occurs first a shallow peristaltic wave followed by a strong antral contraction which is immediately followed by a deep peristaltic wave. The type II contraction is similar to type I. There first occurs a contraction of the antrum but the following peristaltic wave may originate high in the fundus of the stomach, even at or near the cardiac sphincter and sweep over the entire stomach terminating in an antral contraction which may obliterate the lumen of the lower third of the stomach. Type III contractions as studied by the graphic method are characterized by repeated contractions with maintained tonus or increased intra-gastric pressure. This maintained intra-gastric tension, it can be seen fluoroscopically is due to either or both of two factors: first, increased constriction of the antral end of the stomach; and, second, hyper-peristalsis occurring at such a rate that a second peristaltic wave appears before the preceding wave has disappeared. The most striking feature of this work has been the observation of the complete obliteration of the lumen of the antral end of the stomach at the height of the hunger contraction. It is at this time that the subject feels the contraction most intensely.

Decorticate rigidity. F. H. SCOTT.

Most of those who have removed portions of the cerebral cortex have failed to notice decerebrate rigidity and it has come to be generally accepted that rigidity results not from removal of the cerebral cortex, but from transection in the region of the midbrain at or near a supposed rigidity center.

In conjunction with Drs. J. C. McKinley, N. J. Berkwitz and R. E. Morris, a series of animals has been operated upon in which the greater portion of the cortex has been removed. Under anesthesia the calvarium was removed, the dura opened and as much cerebral cortex removed from the hemispheres by scissors as could be done without injuring the remaining brain structures. The plane of removal was very superficial, some cortex

remaining around the deep sulci. In all cases, a well-marked, generalized muscular rigidity appeared on recovery from the anesthetic and the nervous responses to stimuli were apparently entirely reflex.

The rigidities observed differed in detail in the dogs, cats and monkeys examined, but in all cases were postural and responded to stimuli in a similar manner to decerebrate animals. Subsequent section of the brain stem at the level of the colliculi makes little change in the condition provided sufficient cortex has been removed. It appears to us that decerebrate rigidity may well be explained merely on the basis of removal of cortical inhibition.

Gastro-intestinal motility in the guinea pig in experimental scurvy. ERMA ANITA SMITH.

Three methods of study were used:

The emptying time of the stomach after a standard barium meal was determined by means of the x-ray; the rate of passage of food through the gastro-intestinal tract as a whole was measured by feeding a dye and observing the time of initial appearance in the feces; the frequency, amplitude and duration of contraction of isolated segments of intestine in warm oxygenated Locke's solution were measured.

The experiments were conducted upon four groups of twelve animals each. In each group: Four guinea pigs were fed the scurvy-producing diet unsupplemented. These developed acute scurvy and died in about 20 days. Four guinea pigs were fed the scurvy-producing diet plus 0.5 cc. of orange juice daily. These became paralyzed with chronic scurvy and lived in this condition from 4 to 8 months. Four guinea pigs were fed, in addition to the scurvy-producing diet, an adequate amount of orange juice to maintain normal health and vigor. These were the controls.

One hundred and sixty x-ray observations upon the stomachs of 44 guinea pigs were made. Thirty tests upon 16 guinea pigs in acute scurvy showed an average emptying time of 116 minutes. Fifty-six tests upon 12 animals in chronic scurvy showed an average emptying time of 113 minutes. Eighty-four tests upon 16 normal controls showed an average emptying time of 110 minutes. These figures show that the emptying time of the stomach in experimental scurvy is not significantly different from the emptying time in normal control animals.

The dye used in measuring the total time of passage of food through the gastro-intestinal canal was ferric oxide. Tests were conducted simultaneously upon the whole 12 guinea pigs of an experimental group. Twenty tests upon 16 guinea pigs in acute scurvy showed the average interval of passage to be 291 minutes. Eighty tests upon 12 animals in chronic scurvy gave an average of 300 minutes. One hundred twenty-one tests upon 16 controls gave an average of 291 minutes.

The number of tests upon normals total greater because all animals were tested upon the first day of the experiment. This affords a control record on each guinea pig to be added to the record of those kept normal throughout the experiment.

The dye method is a desirable supplement in motility studies because fright and discomfort are ruled out. These are factors of no small consideration in motility studies.

The data presented show that material passes through the gastro-intestinal canal of the scorbutic guinea pig as quickly as in the control animal.

In vitro studies upon isolated segments of intestine were conducted upon scorbutic and control guinea pigs from this series of animals. The details were conducted by Miss Beulah Plummer. Her records reveal no significant motor impairment.

An electrocardiographic study of the embryonic heart (chick). JANE SANDS.

This communication constitutes a preliminary report of a method for taking electrocardiographic records of the chick embryo. It has been possible to obtain records of the heart beat from the thirty-fifth hour on to the end of incubation. The form of the waves seems to alter during the period when the heart is developing from the primitive arteries. Aside from the embryological interest and the possibility of obtaining more exact knowledge as to the development of the heart, the method offers a starting point for the study of the fundamental relations of electrical and mechanical events in relatively simple tissues.

Thermocardiograms from hearts surviving in nitrogen. CHARLES D. SNYDER.

Using a differential thermopile inserted between the split halves of the terrapin's ventricle as before (1922), thermocardiograms were taken from hearts beating alternately for periods now in oxygen and then in nitrogen. The records show that the heat production during diastole and pause (recovery heat production) is affected vastly more by the withdrawal of oxygen than is the heat production during systole (initial or anoxidative heat production). The observation confirms the view held by the author (1922) that the positive waves in the thermocardiograms are an expression of the intrinsic heat production occurring within the heart wall during the chief phases of the cardiac cycle.

Epinephrin content of the suprarenals of thyroidless rats. T. P. SUN.

Male and female albino rats were thyro-parathyroidectomized at 100 days of age. When 150 days old they, and their litter mate controls of the same sex and habitat, were killed by ether. The suprarenals were removed from both tests and controls, and the epinephrin content determined according to Folin, Cannon and Denis, modified to suit the material.

It was found that in 10 of the 15 males, and in 10 of the 16 females, the epinephrin content per gram of suprarenal substance was higher in the glands of the thyroidless than in those of the control rats. The average increase was 24 per cent in the males and 32 per cent in the females.

This finding is consistent with the fact that thyroid deficiency tends to produce cortical shrinkage with consequent increase in relative amount of medullary tissue. This alteration in the proportion of medulla to total gland weight may be the chief cause of the increment, or it may be that the lowered bodily activity of the thyroidless animals is also a participant in the observed reaction.

Constancy of sugar in the sweat of a normal individual. G. A. TALBERT and S. H. SILVERS.

For experimentation the colorimetric method of Folin and Wu was employed for not only testing of the sweat for sugar but also two samples of blood, one of which was drawn just before entering the sweat cabinet and one immediately after retiring from same.

In 28 experiments and 11 different subjects the sweat was found to

contain from 5.6 to 40 mgm. per 100 cc. The latter figure was quite high, the average being near to 15 mgm. per 100 cc.

In 20 instances there was a fall in the blood sugar. In 6 cases an increase while there was 1 without change.

Constancy of amino acids in the sweat of normal individuals. G. A. TALBERT and C. HAUGEN.

The amino acids of the sweat were determined by the same calorimetric method of Folin as in the urine tests.

There were in all 28 experiments on 12 different subjects. In no case did we fail to find amino acids. The concentration ranged from 1.66 to 4.76 mgm. per 100 cc. We were unable to show that there was any constant relationship in the concentration of the amino acids in the sweat and urine. The sweat contains anywhere from one-seventh to one-third the concentration of the urine. The average ratio is about 1 to 5.

The response of smooth muscle in different ionic environments. H. B. VAN DYKE and A. BAIRD HASTINGS.

The response of the guinea pig uterus to given concentrations of carefully standardized pituitary extract was studied in solutions whose ionic composition was varied. Recognizing that the fluids in which the uterus is customarily suspended are not "physiological" we prepared one which was normal with respect to the ions H^+, Na^+, K^+, Ca^{++}, Mg^{++}, Cl^-, $H_2PO_4^-$, $HPO_4^=$, $PO_4^=$, HCO_3^- and $CO_3^=$. A technique was developed for changing at will both the gas bubbled through the solution and the solution itself. The gas consisted of air containing carbon dioxide at a tension calculated to give the desired reaction. Having established the composition required for optimum activity we varied certain of the individual ions; the results obtained with those studied so far are given in the table.

ION VARIED	CONCENTRATION IN NORMAL SOLUTION	DIMINISHED CONCENTRATION	INCREASED CONCENTRATION
	mM per liter		
H^+	$10^{-7.4}$	$10^{-7.8}$: response to pituitrin diminished	$10^{-7.2}$: response to pituitrin increased
Na^+	144.0	87mM: response diminished. Same seen with 30 mM if uterus well oxygenated	
K^+	6.0	3mM: response greatly diminished	9mM: spontaneous contractions; response increased
Ca^{++}	0.5	0.0mM: response almost abolished. 0.3mM: response diminished	0.6mM: response increased 1.0mM: irreversibly increased response
Mg^{++}	1.0	0.0mM: response diminished	2.0mM: response diminished
Total phosphate	1.0	0.0mM: response unchanged	
HCO_3^-	30.0	15mM: response increased	60mM: response decreased

The effect of sweating on the gastric acidity. G. A. TALBERT and I. ROSENBERG.

These experiments were conducted as a rule early in the morning before breakfast, except an Ewald meal, which was given about 20 minutes before entering the sweat cabinet. Immediately following the meal the subject swallowed a modified Rehfus tube which was retained throughout the entire period of experimentation.

Samples of the gastric content were easily drawn without discomfort in 5 seconds of time by means of a suction pump. The samples were drawn regularly at 10-minute intervals which included at least two before entering and two or three after retiring from the sweat cabinet.

From these, tests were made of the free and combined acidity. These results with the total acidity were plotted against the 10-minute intervals of time.

We did forty experiments of which about 85 per cent showed a sudden drop in the free acidity when the subject had been in the cabinet from 20 to 30 minutes. This approximates the time when sweating is the most profuse.

In the 20 control experiments there was followed the same general line of procedure except the sweating was omitted. With the exception of three of these the free acidity curve was more sustained and did not show the characteristic drop of the sweating experiments.

Observations upon the serum calcium after adrenalectomy. N. B. TAYLOR and W. R. CAVEN.

The removal of both adrenals produced a decided rise in the serum calcium within from 3 to 5 hours after the operation. Both adrenals were removed at one operation by the abdominal route. The rise in the calcium varied in different animals from 10 to 40 per cent of the normal. The operation was performed upon some 36 cats, 32 of these (86 per cent) showed the rise. Only four dogs were operated upon; all of these showed a pronounced rise from 12.0 to 15.6 mgm. in one case and from 11.0 to 14.8 mgm. in another. Control experiments in which other organs such as the alimentary canal, kidneys and spleen were removed had no effect upon the serum calcium. Anesthesia was found to have no effect; a decapitated animal in which the adrenals were removed after the effects of the anesthetic had passed off, showed the usual rise. Tying the adrenal veins had the same effect upon the calcium as adrenalectomy. Removal of one adrenal and freeing the other but leaving its blood supply intact was without effect; there was no change in the calcium. Destruction of the glands by crushing had the same effect as complete removal.

In most cases there was a rise in blood concentration accompanying the hypercalcemia; but the increase in blood concentration which was indicated by the hemoglobin estimations, was insufficient in degree to account for the increase in serum calcium. In all cases the percentage of hemoglobin rise was much less than the percentage of calcium rise and in some the calcium rise was unassociated with any increased blood concentration whatever.

Parathyroidectomized animals showed the calcium rise after adrenalectomy, provided the calcium, as a result of the parathyroid removal, had not fallen to the tetany level. If the calcium was depressed to this level (5 to 6 mgm.) adrenalectomy had much less effect in causing it to rise.

In most cases there was no rise at all. This suggests that adrenalectomy affects the calcium level only when there is some parathyroid tissue present. Removal of one adrenal and the meduila of the other was without effect upon the serum calcium.

Extracts prepared from the cortex of ox adrenals depressed the serum calcium in rabbits from 15 to 30 per cent.

The influence of anoxemia on the heart and the rôle of the pericardium in cardiac dilatation. EDWARD J. VAN LIERE.

The normal cardiac area in animals was ascertained by taking x-ray pictures and measuring the area with a perimeter. The animals were then placed in a steel respiratory chamber and subjected to a low atmospheric pressure corresponding to 3.5 to 7.5 per cent oxygen. They were kept in the chamber approximately 8 to 10 minutes and the barometric pressure held at the desired point for 2½ minutes. The animals were then removed and an x-ray picture was taken at once and the cardiac area determined. This was done on normal dogs, a rabbit and a guinea pig and on barbitalized dogs and cats. It was found that there was a dilatation varying from 16.5 per cent to 7.5 per cent depending upon the degree of anoxemia.

In a group of barbitalized cats and dogs the normal cardiac area was ascertained as described above. The pericardium was then slit or practically removed. The animal was allowed to recover from the operation and about two hours later an x-ray picture was again taken. The animals were then subjected to a lowered barometric pressure as described above. Approximately the same degrees of anoxemia were used. It was found that the dilatation which ensued was comparable to that described for the heart with the pericardium intact, that is, if the cardiac area of the heart with the pericardium cut was used for a normal. On the other hand, if the heart with the intact pericardium was used as the normal it was found that the per cent of dilatation was considerably increased. It was noted, moreover, that if the barometric pressure was reduced very rapidly or rather extreme anoxemia was produced a more marked dilatation took place which suggests that the pericardium plays a protective rôle in preventing excessive dilatation in extreme anoxemia.

The rôle of the hypophysis in the initiation of labor. H. B. VAN DYKE and ADOLPH KRAFT.

Dixon and Marshall have published data which seem to prove that the intravenous injection of ovarian extracts from animals at or near term causes the appearance in the cerebrospinal fluid of an oxytocic substance presumably liberated by the posterior lobe of the hypophysis. We have attempted to detect a difference in the concentration of oxytocic substance (by adding cerebrospinal fluids to Tyrode solution baths containing sensitive isolated guinea pig uteri) in the cerebrospinal fluid of patients near term and in labor 3 to 6 hours. In no instance has such an attempt been successful.

The influence of ethyl alcohol upon the oxidative metabolism and the mechanical efficiency of the dog's heart. M. B. VISSCHER.

In confirmation of other workers it has been found that the presence of rather small quantities of alcohol in the circulating blood causes a marked dilatation of the ventricles of the dog's heart in the heart-lung preparation,

when the heart is made to do a constant amount of work. Further, it has been found that the oxygen consumption is directly proportional to the volume of the ventricles, as well when the cardiac muscle has been poisoned by alcohol, as when it is under more physiological conditions. Thus, it is seen that the accomplishment of work is more expensive to the heart when alcohol is present than when it is not. In other words, the mechanical efficiency of the heart is reduced by alcohol poisoning.

The action of ammonium salts. MARY WHELAN, MINARD F. JACOBS and NORMAN M. KEITH.

Following the administration of ammonium salts and their absorption into the blood stream, it has been shown experimentally that some of the ammonia is quickly synthesized to urea. The liberated acid ion increases the anion content of the blood and tissues and causes certain changes in metabolism. The administration of ammonium salts thus offers a method of studying the action of different acid ions. The following salts, ammonium chlorid, ammonium nitrate, ammonium sulphate, ammonium acetate and ammonium benzoate, have been administered to dogs and their effect on certain blood and urine constituents noted. A similar amount of hydrochloric, nitric, sulphuric and acetic acid was given and the action compared to that of the ammonium salt. No toxic effects were noted from the amounts given.

The inorganic acids, benzoic acid and their ammonium salts, produce an acidosis in the tissues, while the acetic acid has no demonstrable effect on the acid-base equilibrium. This latter effect is presumably due to rapid combustion and removal as carbon dioxide by way of the lungs. The nitrate ion appears to have the invariable specific effect of increasing the urinary output of water, and chlorin. This fact offers an explanation for the long known remarkable diuretic properties of nitrates in clinical therapeutics.

A fat formation under abnormal conditions from carbohydrate by the rat, and its relationship to a possible new dietary factor. LAURENCE G. WESSON.

Respiratory quotients as high as 1.5 or 2, indicating fat formation from carbohydrate, were observed after the ingestion of test meals of dextrin by rats that had been subjected to a severely restricted diet for a period of 2 weeks or more. The over-filling of the carbohydrate stores which may cause the normal conversion of carbohydrate to fat is thought to have been avoided by a preliminary fasting interval of 1 or 2 days prior to the test meals. The feeding of small amounts (1 gm. or less) of the ether-soluble substances of certain hog's tissues to rats that gave these abnormal quotients caused normal quotients to be obtained after dextrin test meals fed under the same conditions, while the ether-soluble substances of certain other hog's tissues had little or no effect.

An apparatus and a method for continuously determining the respiratory quotients of small animals were developed for these experiments.

The stimulating effect of amino acids on sugar metabolism and of sugar on amino acid metabolism. MAUDE WILLIAMS and W. E. BURGE.

The amino acids used in this investigation were glycocoll, tyrosin, isoleucin, nor-leucin, cystin and a mixture of all of the naturally occurring amino acids purchased under the trade name of aminoids. The sugars

used were dextrose, levulose and galactose. Benedict's method was used for making the sugar determinations and Van Slyke's as well as Sörensen's for the amino acid determinations. Paramecium caudatum were the animal cells used. Large quantities of these were collected, washed free of debris by centrifugalization and measured in centrifugalizing tubes which were graduated in cubic centimeters. Paramecia sugar preparations were made with 5 cc. of paramecia in 100 cc. of 0.1 per cent sugar solutions. Paramecia amino acid preparations as well as aminoid preparations were also made with 5 cc. of paramecia in 100 cc. of a 0.3 per cent solution of the amino acids as well as the aminoids.

The addition of 300 mgm. of the aminoids to the paramecia sugar preparations greatly increased the utilization of the sugars by the paramecia. In some instances the increase was as much as 75 per cent above the control. The addition of the individual amino acids increased the sugar metabolism only slightly. In fact, nor-leucin decreased it. The addition of 100 mgm. of sugar to the paramecia aminoid preparations as well as the amino acid preparations produced only a very small increase in the metabolism of the aminoids or the amino acids. Hence the stimulating effect of sugar and amino acids on the metabolism of these animal cells is very similar to that of the higher animals for it is known that the amino acids produce the greatest and the sugars the least increase in the metabolism of mammals.

The mode of action of ultra-violet radiation in decreasing sugar metabolism. G. C. WICKWIRE and W. E. BURGE.

The source of the ultra-violet radiation was a Cooper-Hewitt quartz mercury burner, operating at 170 volts and 3.5 amperes. Dextrose, levulose and galactose were the sugars used and paramecium caudatum the animal cells. Benedict's method was used for making the sugar determinations.

A large quantity of paramecia was collected, washed free of debris by centrifugalization and measured in the centrifugalizing tubes which were graduated in cubic centimeters. Paramecia sugar preparations were made with 5 cc. of paramecia in 100 cc. of 0.1 per cent sugar solutions. These preparations were exposed to the radiation at different distances for ten hours and the rate of sugar utilization was compared with that of the controls. It was found that the sugar metabolism in the preparations 50 cm. from the burner was decreased in some instances by as much as 80 per cent. The paramecia in these preparations were killed by the radiation. In other preparations 400 cm. from the burner the sugar metabolism was decreased 35 to 40 per cent. In these preparations the paramecia were not injured so far as appearance was concerned for they appeared as active as those of the controls.

Exposure of insulin to the radiation destroyed it as was indicated by the fact that the injection of the irradiated insulin into rabbits did not produce convulsion whereas the injection of the unexposed insulin did. The addition of insulin from time to time to the irradiated paramecia sugar preparations prevented the decrease in sugar metabolism observed in preparations to which no insulin was added. Hence the decrease in sugar metabolism brought about by ultra-violet radiation is attributed to a destruction of the insulin in the paramecia. Absorption spectra were made of the organisms and it was found that while they did not completely ab-

sorb any of the bands in the short wave length region of the spectrum, they dimmed them, thus showing that the organisms absorbed some of the radiant energy in this region of the spectrum.

(1) *Methods of further purification of pancreatic secretin and* (2) *Occurrence of this substance in the gastro-intestinal tract.* M. M. WEAVER.

(1) It having been found possible to separate the secretagogue fraction from the vaso-dilatin fraction of pancreatic secretin by precipitating the former with sodium chloride, it was desirable to effect a further separation of the active principle from the inert material included in the precipitate.

"New secretin" as prepared by Doctor Luckhardt was used as a starting point, this being saturated with crystalline sodium chloride and the precipitate collected by centrifuging.

The precipitate was redissolved in distilled water and reprecipitated by saturating with sodium chloride, this process being repeated several times.

The precipitate was then thoroughly dried and washed with ether and upon occasion with absolute alcohol or absolute acetone or both.

The precipitate was then extracted twice with 95 per cent alcohol, or with 95 per cent acetone, alcohol giving the best results. The alcoholic or acetone extract was then dried in air or by gentle heat and the residue taken up in physiological saline for injection. The amount of residue upon evaporation of the solvent may be minute in quantity but of high potency as a stimulant of pancreatic secretion.

(2) Buccal cavity, esophagus, cardia of stomach, fundus of stomach, duodenum, the small intestine by fifths and the colon and rectum were extracted with 0.4 per cent HCl by the general method of preparing "new secretin," dogs being used.

Activity was found in the duodenal extract and in rapidly decreasing quantities down to but not including the last fifth of the small intestine. Rarely a slightly active preparation was obtained from the fundus of the stomach. All other extracts were negative as were extracts from peritoneal cavity, pleural cavity, large blood vessels, liver, cardiac muscle and a variety of other tissues. Many of the extracts in the latter category and especially those of colon and stomach gave a considerable precipitate upon addition of NaCl but this precipitate was found to be inactive.

Some factors governing renal function. BURNHAM SARLE WALKER.

The author has studied the rate of elimination of urea, as compared with the amount of urea in the blood and with the simultaneous excretion of water.

In regard to the relationship of blood urea level to rate of urea excretion, it will be recalled that there are two divergent schools of thought. The older group, of which Ambard and McLean are the outstanding examples, have shown that the rate of excretion is a parabolic function of the concentration of urea in the blood, according to the equation $Ur = K\sqrt{D}$. Ur = gram urea per liter blood. D = gram urea per 24 hours. Addis, and later Adolph, on the other hand, under conditions involving a large provocative ingestion of either urea or water, or both, have demonstrated a linear relationship, $Ur = KD$.

By combination of the author's data with those of the above, the function is found to be a sub-parabola lying intermediate between the parabola of Ambard and the line of Addis.

Over the range of the values of blood urea usually found, this relationship varies from that proposed by Ambard by less than the ordinary variations of either. Assuming a constant of 0.10 in the Ambard equation defines a limit dividing the values found in normals, or incipient nephritics, from those found in the more advanced stages of the disease. This curve includes 96.5 per cent of the normal cases. A similar limiting curve drawn on a basis of the Ambard equation, with a constant of 0.075, gives us a basis of division between the nephritics of all grades and the normal, and includes 96 per cent of all the nephritic cases studied. The area intermediate to these two curves is an indeterminate area, with a suspicion of pathology.

To simplify calculation, we have altered the original Ambard formula by inverting it and adding a coefficient 7.5, which permits us to group our subjects on the scale of 100, in accordance with the McLean convention. This final form of the Ambard expression is $7.5 \frac{\sqrt{D}}{Ur} = F$—functional capacity of kidney on scale of 100. The following table gives a summary of the distribution of our cases on this basis:

	NORMAL CASES	CASES WITH RENAL IMPAIRMENT
	per cent	*per cent*
F above 100 (high normal group)	76	4
F between 100 and 75 (low normal or doubtful group)	20.5	56
F below 75 (deficient group)	3.5	40

This expression differs from the Ambard formula and the McLean index in that it neglects the correction factors for body weight and urine concentration. These factors have been found to be misleading both by the author and previous investigators. The present formula offers a method for evaluating the efficiency of the kidney in respect to the removal of urea from the blood stream under the normal conditions of daily life, uncomplicated by those additional variables.

The importance of dynamic factors in ventricular alternation. CARL J. WIGGERS.

The assumption that the large as well as the small beat of an alternans couple is abnormal is basic to several theories of ventricular alternation. Experiments on ventricular alternation in the dog's heart produced by rapid stimulation of the auricle do not bear this out. Intraventricular pressure curves showed that the amplitude, gradient and duration of the large beat of an alternans couple were usually identical with normal beats occurring at the same rate. Occasionally, however, it was of slightly larger amplitude, rose more steeply and had a longer duration of contraction. These observations make it improbable that a smaller number of fractions contract in the large beat of such an alternans couple than in a normal beat occurring at the same heart rate.

The increased size of the larger beat may be explained by assuming that a state of potential alternation had existed in the apparently non-alternating beats; on the other hand, it may equally well be explained by a slightly greater initial length and tension which preceded the larger beat.

Another series of experiments lends further support to the possibility that rhythmic variations in diastolic size may be a primary and only cause of certain forms of ventricular alternation. When a dog's heart is beating at a rate above the critical level of about 140 per minute, a condition of temporary alternation is induced by any condition which produces a prolonged diastolic pause (i.e., by ventricular or auricular premature systoles, temporary a-v block, or brief vagal stimulation).[1] In such cases the alternation appears to be definitely induced by dynamic changes. Records show that, as a result of the prolonged diastolic pause, ventricular filling is greater and initial tension is increased. In response, the ventricle not only ejects this larger volume but in addition empties itself more completely. It does this by increasing the velocity of ejection and by prolonging the phase of ejection. This does not delay the opening of the a-v valves and the beginning of filling, because the isometric relaxation rate is more rapid. During the early phase of filling, blood flows in from the auricle at somewhat greater velocity, but this is not sufficient to fill the more completely emptied ventricle to natural diastolic size during the interval available. A smaller beat during which the ventricle is less completely emptied results. The retention of this blood, together with a normal inflow volume, again acts, however, to fill the ventricle to a greater extent, with the result that a larger contraction follows, etc.

In view of these experimental observations, our present view that ventricular alternation can not occur without primary involvement of cardiac muscle should be submitted to further investigation.

On the specificity of pancreatic secretin. M. M. WEAVER.

It has been demonstrated that pancreatic secretin preparations after the method of Bayliss and Starling may stimulate the gastric glands to secrete and in addition may induce a flow of saliva from the salivary glands and of bile from the liver.

Using purified secretin preparations for injection of Pavlov dogs, and a Heidenhain pouch dog with permanent fistula of the lower pancreatic duct, it has been impossible to demonstrate any increase in flow of gastric juice, whether such injections be subcutaneous, intramuscular or intravenous.

The permanent pancreatic fistula has upon several occasions been caused to secrete a copious amount of pancreatic juice, this occurring most markedly after intravenous injection of the secretin, the gastric pouch remaining refractory.

Vagus inhibition of the fall in blood pressure in peptone shock. RUSSELL A. WAUD.

In a previous paper (Waud, 1926) the theory has been advanced that a sudden reduction in the viscosity of the blood is an important factor in the production of the fall in blood pressure in peptone shock. The present work in which 25 rabbits were studied lends support to the same view.

Under urethane anesthesia blood pressure was recorded by the usual method, and the peripheral end of the cut vagus was prepared for stimulation. While the blood pressure was maintained at a low level by vagus

[1] A detailed report of these experiments will be published in the Warthin Anniversary Volume, George Wahr, Ann Arbor, Michigan (in press).

inhibition, 0.5 to 1.0 gram per kilo of body weight of "Witte" peptone was injected into a vein. Instead of the prolonged depression of blood pressure characteristic of the injection of this quantity of peptone, it was found that on removing the vagus effect the blood pressure immediately returned to normal or above and remained there.

It is probable that these results may be explained as follows: *a*. The slowing of the circulation may allow the peptone to remain in some organ longer, and thus is more completely destroyed in that organ. *b*. The effect may be due to the return of the viscosity of the blood to normal, while the blood pressure in the arterioles and capillaries is maintained at a low level. As communicated previously it is believed that the fall in blood pressure in shock is brought about by a sudden reduction in the viscosity of the blood, and thus a reduction in the peripheral resistance. During the fall in blood pressure a large volume of blood under excessive pressure is allowed to enter the capillaries. The capillaries being injured beyond immediate repair are unable to contract and return the blood to the current circulation. Thus the blood pressure remains low beyond the period of reduced viscosity. Vagus stimulation may exercise its inhibitory effect by keeping the pressure in the large arteries at a low level until the transitory period of low blood viscosity is passed.